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BEARING ON HEREDITY AND VARIATION

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J. Talbot Hall

J. ARTHUR HARRIS

JAMES ARTHUR HARRIS was born September 29, 1880 in Plantsville, Athens County, Ohio, migrated with his family to western Nebraska, then to Kansas. He studied, 1899–1901, at the University of Kansas, where he came under the influence of Professor C. E. McCLUNG; was appointed (1901) an assistant in the Missouri Botanical Garden, where he came under the influence of WILLIAM TRELEASE, and later (1903–07) served as its librarian. In 1907 he became Resident Investigator at the Carnegie Institution's Station for Experimental Evolution at Cold Spring Harbor, continued there until 1924 and published while there about 250 papers. At the beginning his attention was directed toward variation, adaptation, natural selection and biometry, to which latter methodology his contacts with KARL PEARSON in London (1908–09 and 1909–10) gave special stimulus. Beginning in 1911 he studied the physico-chemical proportions of cell saps as a factor influencing plant distribution and this investigation led to trips to Virginia, Florida, Jamaica, Honolulu and the Southwest. In 1924 he was called to the professorship of botany and biometry at the University of Minnesota. He died, on April 24, 1930, at St. Paul, Minnesota.

HARRIS' contributions to genetics in the narrower sense were of two types. First, the experimental. HARRIS' experiments were at first largely devoted to testing the theory of natural selection. Thus he showed that in Staphylea there is selective elimination of ovaries resulting in an increase in mean number of ovules; also the proportion of ovaries with odd number of ovules per locule is cut down. Then he reared *Phaseolus vulgaris* from ancestors that had been starved for a generation or two, but found little difference in the number of pods per plant between the starved line and the controls. But when heavy seeds were planted they produced more pods per plant than less heavy ones. Also, in the seedlings there is a selective death rate which tends to reduce variability in the weight of viable seeds of the next generation. These studies on beans involved thousands of plants and the weighing of nearly 100,000 individual seeds. In this work a polycotyledonous strain was observed.

Second, the biometrical. The influence of PEARSON on HARRIS was great and he became one of the most original biometricians of his time in America. He proposed useful methods of shortening biometric calculations especially with the use of machines. His ideal of collecting thousands of individual data for each biometric calculation was carried to an extreme degree and because he separated intra class and inter class data they have been immediately useful in the analysis of variance at the hands of others. His facility in biometric analysis was utilized in the researches of his second

period; but, before that, in his classical joint book with F. G. BENEDICT "A Biometrical Study of Basal Metabolism in Man." To this biometric period belong his studies upon correlations: between a variable and the deviation of a dependent variable from its probable value; between egg production in fowl and body pigmentation and period of the year and the correlations between successive periods of plant growth. He also analyzed extensive data concerning human births and the sex ratio. Biometric analysis accompanied all of his later work.

The second period of HARRIS' scientific career may be said to have begun with his association at the Station for Experimental Evolution with DR. R. A. GORTNER, in 1913, and the interest in stratum-heterogeneity growing out of his extensive breeding of beans. Together they worked out the technique of determination of the osmotic pressure by the depression of the freezing point of vegetable saps and this led to wide-ranging travels in making the new application of this method. His last publication (1930, posthumous) is a work of over 300 pages on the physico-chemical properties of plant saps in relation to phytogeography.

Not less striking than the enormous literary and scientific prolificacy of HARRIS were his personal qualities. He was not above medium stature, was somewhat stocky, and suffered from poor eyesight. He readily became so submersed in work as to be oblivious to all else. In his relaxed periods at home and in camp he showed his rare sense of humor and his capacity for mimicking others. He has left some striking examples of parody and sarcasm like his address on "A Great Institute for the Study of the Mule." His home life was exceptionally happy, with Emma Lay Harris and their four sons.

After his death there was published in book form a biographical tribute edited by his University associates: C. O. ROSENDAHL, ROSS A. GORTNER and GEORGE O. BURR (University of Minnesota Press, 1936). This contains five hitherto unpublished papers, in the preparation of which his assistants have joined. It includes nine illustrations, mostly of DR. HARRIS and party in the field.

C. B. DAVENPORT

The frontispiece photograph is from "J. Arthur Harris, Botanist and Biometrician" and is copyrighted by the publishers, University of Minnesota Press. It is reproduced by courtesy of the Press.

* * *

LOCALLY SPECIFIC PATTERNS OF CHROMATID AND CHROMOSOME INTERFERENCE IN NEUROSPORA[1]

CARL C. LINDEGREN AND GERTRUDE LINDEGREN
University of Southern California

Received May 3, 1941

INTRODUCTION

ONE of the fundamental problems concerning the mechanism of crossing over is the question of chromatid interference. In the absence of chromatid interference the identity of the chromatids involved in two or more chiasmata or points of crossing over is random. Positive chromatid interference would exist if a crossover between one pair of chromatids reduced their chances of being involved in a second crossover with a resulting excess of 4-strand exchanges. Negative chromatid interference would exist if there was an excess of exchanges over that expected on a random basis in which a crossover between one pair of chromatids was followed by a second crossover between the same pair (2-strand exchange). The present paper and previous ones (LINDEGREN and LINDEGREN 1937, 1939) describe chromatid interference (non-random crossing over) in Neurospora. The conclusions reached in these studies are not in agreement with those of BEADLE and EMERSON (1935) on Drosophila. HUSKINS and NEWCOMBE (1941), however, demonstrated chromatid interference in Trillium by direct cytological observation. NEWCOMBE (1941) after a statistical analysis of BEADLE and EMERSON'S (1935) data, questions the validity of their interpretation. BONNIER and NORDENSKIÖLD (1937) failed to corroborate BEADLE and EMERSON's findings.

Our data show that when two crossovers occur symmetrically spaced on each side of the centromere, the same pair of strands is usually involved in each of the two exchanges, but in double exchanges involving certain other pairs of regions, the first exchange may interfere with a second exchange occurring between the same two chromatids. A pattern of exchanges involving negative chromatid interference between certain pairs of regions is associated with a pattern involving positive chromatid interference between other pairs of regions. There is a specific pattern characteristic of each pair of regions, and an excess of one type of exchange may occur between one pair of regions with a deficiency of that same type between another pair of regions.

Directly related to the question of chromatid interference is the question of chromosome interference. If chromosome interference is positive,

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an exchange in one region inhibits an exchange in an adjacent region, and the observed number of multiple exchanges is less than the calculated number. We found a marked excess of multiple exchanges and at the same time a distribution of positive chromosome interference between certain pairs of regions, and negative chromosome interference between other pairs of regions. Tetrad analysis shows a pattern of chromosome interference around the centromere which is not entirely dependent on the distances of the regions from each other, but more on a basic pattern specific for the different pairs of regions.

Standard techniques for calculating chromosome interference or predicting the expected number and types of exchanges depend on the basic assumption that chromatid interference does not exist. Our data indicate that chromatid interference is variably distributed along the Neurospora chromosome. Therefore, the standard methods of calculating coincidence based on the assumption that chromatid interference does not occur are invalid if chromatid interference exists as it does in Neurospora.

EXPERIMENTAL

Comparison of Two Experiments

In a series of earlier papers, the technique of using the ascomycete Neurospora for genetical analysis has been described. LINDEGREN and LINDEGREN (1937) analyzed hybrid asci from a cross between a (+) gap-pale stock and a (−) crisp stock of *Neurospora crassa*. The locations of these genes on the sex chromosome are shown in figure 1. These stocks were relatively infertile and were inbred by LINDEGREN, BEANFIELD, and BARBER (1939) to increase fertility. Selection was successful, and experiments were reported describing an increase from 5 to 70 percent fertility in four generations. Subsequent to publication, inbreeding was continued for about seven more generations, and stocks were obtained which produced ascospores every time (+) and (−) cultures were grown together. From this inbred collection (+) gap-pale and (−) crisp stocks were mated; table 1 contains the data on this and a previously published experiment. The 494 asci isolated in 1937 contained 6.5 percent of asci in which multiple exchanges occurred, while in the 1940 series only 3.7 percent of multiple exchanges were found. This difference may be due to the fact that the second experiment was done in the winter time when the laboratory was cooler, while the first experiment was done in the summer when the higher temperatures might have increased the amount of crossing over. Although the frequency of exchange is greater in the 1937 data, the kinds of exchanges which occurred are similar in both experiments. The 1937 and 1940 data were calculated separately to compare the expected frequency of multiple exchange with the observed frequency. The different kinds of ex-

changes (from the 1937 and 1940 data) were collected into homogeneous groups in order to compare the observed with the expected values of chromatid interference.

In another respect the two experiments are different. A majority of the cultures grown in 1937 were tested to determine the distribution of the sex genes among the progeny. This character can only be determined by a breeding experiment. This is much more time-consuming than the technique of classifying the progeny with regard to gap, crisp, and pale by inspection. In collecting the 1940 data, only cultures from those asci showing crossing over in regions II, III, or IV were tested for sex to determine

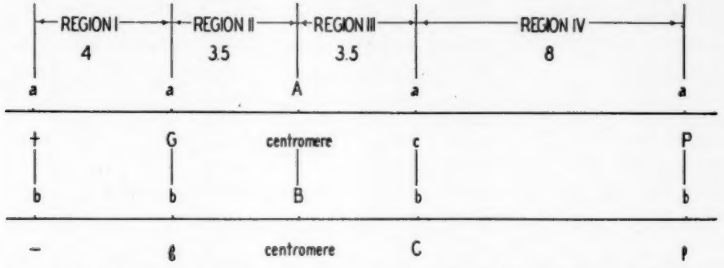


FIGURE 1.—Regions, distances, and gene-markers in the two homologous chromosomes. The (+) gap-pale chromosome is the "a" chromosome with the respective genes (including non-crisp, *c*) designated by small letter "a" arranged in the same order as the genes. The centromere is indicated by large A. The (–) crisp chromosome is the "b" chromosome with the respective genes indicated by small letters "b" arranged in the same order as the respective genes (including non-gap, *g*, and non-pale, *p*).

if crossing over had also occurred in region I. The number of single exchanges in region I was calculated from what was known to have occurred in 1937. In only 225 (1937) non-crossover asci were complete sex tests available. There were 15 complete asci (otherwise non-crossovers) showing second division segregation for sex. An additional 123 asci which had not been tested for sex were otherwise non-crossover asci. This increased the total for 1937 over that previously published. To summarize:

tested for sex.....225 non-crossover asci
tested for sex..... 15 single crossovers in I
not tested for sex.....123 non-crossover asci

363

To calculate the amount of single crossovers in region I

225:15::363:X X=24
non-crossovers 363–24=339

TABLE 1

Analysis of the different types of exchanges by the arrangements and genotypes of the ascospore cultures.

EXCHANGES REGIONS	STRANDS	GENOTYPES OF SPORE PAIRS				NUMBER OF ASCI		
		1&2	3&4	5&6	7&8	1940	1937	TOTAL
None		aaAaa	aaAaa	bbBbb	bbBbb	711[c]	339[c]	1050[c]
Single I		aaAaa	baAaa	abBbb	bbBbb	73[c]	24[c]	97[c]
Single II		aaAaa	bbAaa	aaBbb	bbBbb	54	18	72
Single III		aaAaa	aaAbb	bbBaa	bbBbb	61	16	77
Single IV		aaAaa	aaAab	bbBba	bbBbb	142	63	205
Double I–II	2	aaAaa	abAaa	baBbb	bbBbb	1		1
Double I–III	2	aaAaa	baAbb	abBaa	bbBbb	2		2
Double I–IV	2	aaAaa	baAab	abBba	bbBbb	5	10	15
Double II–II	2	aaAaa	bbAbb	aaBaa	bbBbb	9	1	10
Double I–III	3	aaAaa	baAbb	abBbb	bbBaa		1	1
Double I–III	3	aaAbb	baAaa	abBaa	bbBbb		1	1
Double I–IV	3	aaAaa	baAab	bbBba	abBbb	1	4	5
Double I–IV	3	baAaa	aaAab	abBba	bbBbb	1	1	2
Double II–III	3	aaAbb	bbAaa	aaBaa	bbBbb	2		2
Double III–IV	3	aaAba	aaAab	bbBaa	bbBbb	2		2
Double I–II	4	baAaa	bbAaa	aaBbb	abBbb	1	1	2
Double I–IV	4	aaAab	baAaa	abBbb	bbBba	2	4	6
Double II–III	4	aaAbb	bbAaa	aaBbb	bbBaa		1	1
Double II–IV	4	aaAab	bbAaa	aaBbb	bbBba	4		4
Double III–IV	4	aaAab	aaAbb	bbBaa	bbBba	2		2
Double II or III	4	aaAbb bbAaa	aaAbb bbAaa	bbBaa aaBbb	bbBaa aaBbb	1		1
Double IV	4	aaAab	aaAab	bbBba	bbBba		1	1
Triple I–II–III	2-2	aaAaa	abAbb	baBaa	bbBbb		1	1
	4-2	baAaa	bbAbb	aaBaa	abBbb			
Triple I–II–III	3-2	abAbb aaAaa	baAaa bbAbb	aaBaa abBbb	bbBbb baBaa		1	1
Triple I–II–IV	4-4	baAab	bbAaa	aaBbb	abBba		1	1
Triple I–III–IV	3-4	aaAab	baAbb	bbBaa	abBba		2	2
Triple I–III–IV	4-4	aaAbb	baAab	abBba	bbBaa	1		1
Triple II-III–IV	2-3	aaAaa bbAba	bbAbb aaAab	aaBab bbBbb	bbBba aaBaa		1	1
Triple II–III–IV	2-2 2-4	aaAaa aaAab	bbAba bbAbb	aaBab bbBba	bbBbb aaBaa		1	1
Triple I–IV	4-2, 4-3, 4-4	baAaa	baAab	abBbb	abBba	1		1
Quad. I–II–III–IV	2-2-3 4-2-3	aaAaa bbAba	abAbb baAab	bbBba aaBaa	baBab abBbb	2	2	4
Quad. I–II–III–IV	2-2-2 4-2-4	aaAab baAaa	abAba bbAbb	baBaa aaBab	bbBbb abBba	1		1
Quad. I–II–III–IV	3-3-3	baAbb	abAaa	aaBab	bbBba	1		1
Quad. I–II–III	4-2-2 4-4-2	abAbb abAbb	baAaa baAaa	abBbb abBbb	baBaa baBaa	1		1
						1081	494	1575

c = Calculated

In the 1940 data there were 784 non-crossover asci which were not tested for sex. The number of single exchanges in region I was calculated as follows: The average of the ratios of single exchanges in the different regions —SII(1940)/SII(1937), SIII(1940)/SIII(1937), SIV(1940)/SIV(1937)— was obtained as follows: $54/18 + 61/16 + 142/63 \div 3 = 3.02$. $24 \times 3.02 = 73$.

The number of single exchanges in region I, calculated for the 1940 data, is 73. It seemed more reasonable to calculate the single exchanges in region I on the basis of the singles in II, III, and IV than on any other basis, but the difference between the two experiments makes any calculation of this type subject to error.

These calculated values enter into the comparison of the expected and observed frequencies of multiple exchanges. However, the comparison of the different kinds of multiple exchanges is unaffected by the necessity of calculating the frequency of single exchange in region I. The conclusions concerning chromatid interference are independent of this calculated value.

In addition to adding the asci which had not been tested for sex to the 1937 data, a few asci from which too few spores were obtained were discarded and others were reclassified on the basis of certain indeterminacies discussed below. Some numerical errors were corrected, and this table supersedes that originally published in 1937.

System of Representing Chromatids

Table 1 has been simplified by designating all the genes in the (+) gap-pale chromosome by the letter a: the first letter (a) represents (+), the second (a), gap; the third letter (*A*) represents the centromere, the fourth letter (a) represents the normal allele of crisp, and the fifth letter (a) represents the gene, pale (fig. 1). In the homologous chromosome, the letter b is used to represent its marked loci as follows: the first letter (b) on the left represents the (−) locus, the second letter (b) represents the normal allele of gap, the third letter (*B*) represents the centromere, the fourth letter (b) represents crisp, and the fifth (b), represents the normal allele of pale. This system makes it easy to distinguish crossovers. For example, b a A a a, represents (−) gap-pale and indicates that an exchange has occurred in the region between (+) and gap.

The relation of the position of the spores in the ascus to crossing over between the chromosomes has been considered in detail in an earlier paper (LINDEGREN and LINDEGREN 1937). Figure 2a shows the arrangement resulting when no crossovers occur between the (+) gap-pale and the (−) crisp chromosome. The two reduction divisions occurring in the long narrow ascus arrange the spores in order. Four spores at one end are (+) gap-pale while the other four spores are (−) crisp. When a crossover occurs in region II (fig. 2b) the chromatids attached to the A centromere

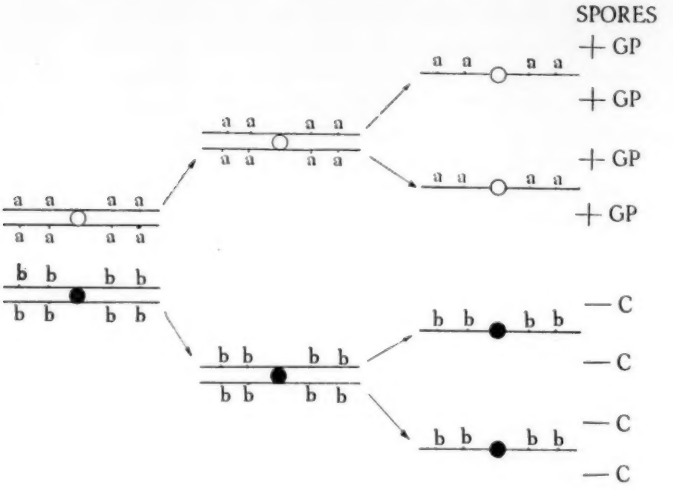


FIGURE 2.—(a) Diagram showing the arrangement of the spores in the ascus when no crossing over occurs in the marked regions. (b) Diagram showing the change in linkage and the arrangement of spores when a single crossover occurs in region II.

are respectively (+) gap-pale and (−) pale (a a A a a and b b A a a). The chromatids attached to the B centromere are respectively a a B b b and b b B b b, (+*GC* and −*C*). A final arrangement of spores like that shown in figure 2b may occur. Alternative arrangements depend on the orientation of the spindles at the three divisions, and these alternative arrangements are also interpreted as the result of a crossover in region II.

This technique uses the centromere as a locus. The map distance between the centromere and the locus is one-half the percentage of second-division segregation. This is clear from a study of figures 2a and b. For example, if second-division segregation of gap occurs in seven percent of the asci (2b), the percentage of crossover chromatids (−*P*, +*GC*) is 3.5, and the distance between the centromere and gap is 3.5.

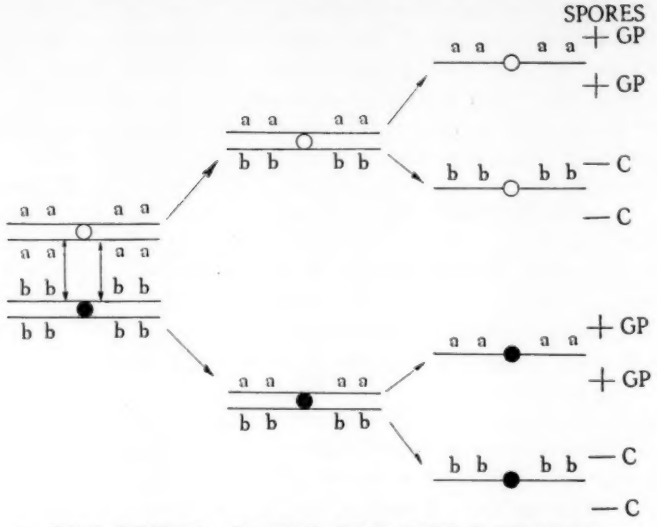
In using the centromere as a locus, it is necessary to establish the fact that there has been no overlapping of second-division spindles which would produce a false picture of second-division segregation if only the arrangements in the ascus were considered. This results from the fact that the centromere is not a marker for any phenotypic character. In figure 3a, a double exchange which resulted in second-division segregation of all the genes is shown. It is compared with a similar arrangement (3b) which might possibly occur if the spindles overlapped at the second division. The possible overlapping of the second division spindles will be considered later.

Classification of Progeny

The characters gap, crisp, and pale and their combinations are determined by inspection of the haploid cultures derived from the ascospores. The combinations such as gap-pale, crisp-pale, and gap-crisp-pale are readily distinguishable. Gap-crisp resembles crisp closely, differing only by a relatively delicate character. Nevertheless, it can be distinguished with experience, for the yellow substrate color of gap is clearly evident in the gap-crisp combination. When all eight spores germinate, it is possible to check a doubtful classification by elimination. For example, consider ascus 3377 (table 2). Since gap, crisp-pale, and pale are easy to classify, the genotype of spores 3 and 4 must be gap-crisp. This means that it would be possible to determine the genotypes of spores 3 and 4 in ascus 3377 even if they had not produced cultures.

Spore Germination

Every spore did not always germinate, so a description of the cultures obtained from the critical groups of triple and quadruple exchange asci is presented in detail to demonstrate the validity of the classification (table 2). It was obviously not feasible to consider all the 1081 asci in similar de-



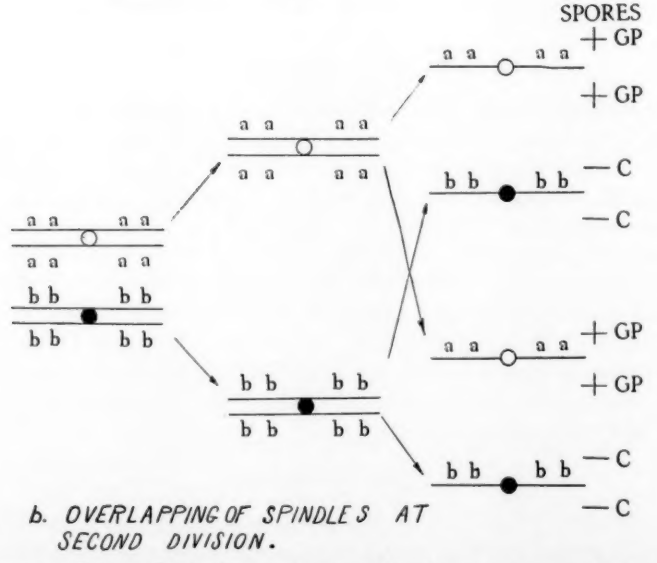


FIGURE 3.—Diagram showing how overlapping of the spindles at the second division could produce an arrangement of spores in the ascus indistinguishable from a 2-strand double exchange between regions II and III, because the centromere does not mark any phenotypic character.

TABLE 2

Arrangements and genotypes of ascospores from triple and quadruple exchange asci.

TRIPLE	REGIONS	YEAR	SPORE NUMBER							
			1	2	3	4	5	6	7	8
3243	I–II–III	37	*+C*		*GP*		*−C*	*C*	*+GP*	*GP*
2968	I–II–III	37	*−C*	*C*	*−GP*	*GP*	*+C*	*C*	*+GP*	*GP*
3150	II–III–IV	37	*−CP*		*GP*	*+GP*	*−C*	*−C*		*+G*
3249	II–III–IV	37	*−C*	*C*	*+GP*		*−CP*	*−CP*	*+G*	*G*
3320	I–II–IV	37	*−P*	*P*	*−G*	*G*	*+GC*	*GC*	*+CP*	
3377	I–III–IV	37	*G*	*+G*	*−GC*	*GC*	*+CP*	*CP*	*P*	*−P*
3400	I–III–IV	37	*+CP*		*−P*		*G*	*+G*	*−GC*	*−GC*
6197	I–III–IV	40	*−P*			*+GC*	*CP*	*+GC*		*−G*
6273	I–IV	40	*−GP*	*G*		*−G*		*+C*	*CP*	*+CP*
Quadruples										
3361	I–II–III–IV	37	*+C*	*C*		*+GP*		*−CP*	*−G*	
2958	I–II–III–IV	37		*+GP*	*+C*	*C*	*−CP*	*CP*	*−G*	*G*
6091	I–II–III–IV	40	*+CP*	*CP*	*+G*	*G*	*−GP*	*GP*		
6129	I–II–III–IV	40	*−G*	*G*	*C*		*+GP*	*GP*	*+CP*	
6132	I–II–III–IV	40	*+C*	*C*	*+GP*	*+GP*	*−G*	*G*	*−CP*	*CP*
6283	I–II–III	40	*+C*	*−GP*	*+C*	*−GP*	*+C*	*C*		*−GP*
7162	I–II–III–IV	40			*+P*	*P*	*+G*	*G*	*−CP*	*CP*

tail, but the same general principles have been used in identifying the genotypes of the absent spores. Asci whose analysis was uncertain because an insufficient number of spores had germinated were discarded. However, it was often possible to classify an ascus by the identification of three spores. For example, −C in positions 6, 7, and 8 definitely establishes a non-crossover ascus.

Eighty-three percent of the ascospores from the asci listed in table 2 produced cultures. This is a higher germination than commonly found.

Spore Abortion

The 1940 experiments were performed with stocks that always produced perithecia and ascospores every time (+) and (−) mycelia were grown together, but in many of the asci each of the eight spores had degenerated to a fat globule. This condition of total ascus abortion is found in some

The arrangement (a) below is due to a 2-strand double exchange between II and III, but it is not distinguishable phenotypically from (b) which is due to overlapping of the spindles.

	aaAaa		aaAaa
(a)	bbAbb	(b)	bbBbb
	aaBaa		aaAaa
	bbBbb		bbBbb

The question is discussed at length in the text and it is concluded that overlapping is rare, if indeed it exists at all.

asci of all Neurospora cultures. Although the cause is not known, total abortion is apparently influenced by both environmental conditions and genetic factors. It is assumed that the asci eliminated by total abortion are selected at random with respect to crossing over in the sex chromosome. This question (which obviously could not be tested in the case of total abortion) was tested in the case of partial ascus abortion. In a small proportion of the asci some of the ascospores are aborted while others are viable. It was possible to prove that this type of partial abortion occurred at random with respect to the type of crossing over occurring in the sex chromosome.

The data presented in table 1 were all obtained from asci in which all eight spores were filled out and dark colored, the conditions associated with ripeness and viability. Only about 75 percent of these spores germinated to produce cultures, and about 30 percent of the asci were discarded because too few spores germinated to permit accurate classification.

Transposition of Spores

In dissecting the spores from the ascus, occasionally two spores—for example, 4 and 5—may be removed together. Then it is sometimes difficult to be certain of their identity. Furthermore, the asci lose their elasticity as they grow older, and adjacent spores may slip around each other when dissection is made. Either one or the other of these events occurred in asci 6283, 6197, and 6273 (table 2). It is clear that by transposing 4 and 5 in 6197, a normal arrangement is obtained. Three cases of transposition are shown in table 2 (16 asci). This is higher than is usually found. More spores must be grown from a multiple exchange ascus to classify it. In a non-crossover ascus a 2-3 or 6-7 transposition is not detectable.

One member of each pair of spores was tested for sex by mating with a (−) mycelium. The absence of a sign indicating sex in table 2 indicates that that culture was not mated to a tester strain. Some of the cultures were relatively infertile—that is, produced only a few perithecia and ascospores when mated to a standard tester strain. Often more than one test was necessary to determine sex. Since there are always four (+) spores found in each ascus, two positive tests of two different genotypes are sufficient to classify the ascus. In each ascus in table 2, two of the four genotypes are (+), so no error in classification resulted from infertility.

Determination of Type of Exchange

A simple rapid method of determining the type of exchange has been developed. For example, ascus 3243 (table 1, lines 26 and 27; table 2) contains the following genotypes: a b A b b, b a A a a, b b B b b, a a B a a. It is important to observe that spores 1, 2, 3, and 4 are shown as containing

centromere A, while spores 5, 6, 7 and 8 are shown to contain centromere B. This is done on the basis of the assumption that if spores 1, 2, 3, and 4 contain the centromere A, spores 5, 6, 7, and 8 will contain B and *vice versa*. This is an application of the assumption of first division segregation of the centromere. It is not possible in this particular ascus to distinguish between two possibilities—namely, whether spores 1, 2, 3, and 4 contain A or B centromere. This is because a 2-strand double exchange has taken place in regions II and III. Such a crossover simply exchanges the centromeres between two chromatids without changing the order of any phenotype-producing genes; therefore, two possibilities are presented.

SPORE NO.	CHROMATID	FIRST POSSIBILITY	SECOND POSSIBILITY
1 & 2	first	a↑ b↑ A↑ b b	a↑ b B b b
3 & 4	second	b↓ a A a a	b↓ a↑ B↑ a a
5 & 6	third	b b B b b	b b↓ A↓ b b
7 & 8	fourth	a a↓ B↓ a a	a a A a a

In the first interpretation, the first chromatid is a triple crossover, the second a single, the third a non-crossover, and the fourth a double crossover chromatid. Arrows are then drawn to connect the chromatids at points where crossovers have occurred. The first double exchange in regions I and II is a 3-strand exchange because it involves the first, second, and fourth chromatids. The second double exchange is a 2-strand exchange in regions II and III. Only the first and fourth chromatids are involved in this exchange. In either interpretation the same conclusion is reached: a 3-strand exchange occurred in regions I and II, and a 2-strand exchange occurred in regions II and III. A multiple exchange also occurred in regions I and III, but only adjacent exchanges have been classified in this work. If the spindles at the second division had overlapped in ascus 3243, the arrangement of the unmarked centromeres might be that shown below:

a ↑ b B b b

b ↓ a A a a

b b B b b

a a A a a

This changes a 3-strand, 2-strand triple exchange in regions I, II, and III, to a single exchange in region I. It will be shown subsequently that overlapping of the spindles occurs rarely or not at all.

Many multiple exchange asci are not changed with respect to the interpretation of crossovers which had occurred by any rearrangement of spores in the ascus. In fact, only those asci in which 2-strand double exchanges occur in regions II and III or those in which a 4-strand double exchange occurs in either II or III are affected. In ascus 6197, there was a transposi-

tion of spores from 4 to 5, but analysis of the crossing over shows a 4-strand exchange in regions III and IV. No matter how the spores are arranged in this ascus, the series of crossovers would not be affected. No amount of transposition would change its classification.

Indeterminate 2- or 4-strand Exchanges

We are indebted to MR. EVERETT DEMPSTER and DR. STERLING EMERSON for pointing out to us that in some cases it is not possible to distinguish 2- from 4-strand double exchanges. The two papers published by us in 1937 and 1939 were in error in not recognizing this possibility. For this reason, the entire data are recalculated in this paper. This error produced very little change in the 1937 results. Ascus 3150 is typical: $-CP$, $+GP$, $-C$, $+G$. Arranging the genotypes according to two alternative schemes because of the indistinguishability of the centromeres.

b	b	A	b	a
a	a	A	a	a
b	b	B	b	b
a	a	B	a	b

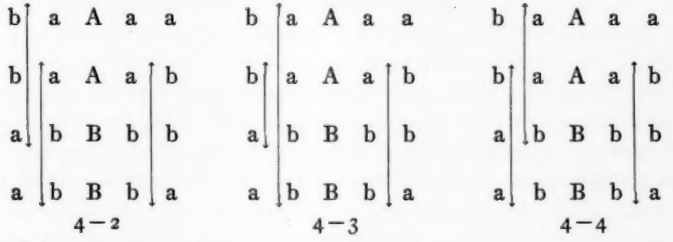
b	b	B	b	a
a	a	B	a	a
b	b	A	b	b
a	a	A	a	b

The first arrangement shows a 2-strand double exchange in regions II and III, followed by a 2-strand exchange in regions III and IV (2-2 triple II, III, IV). The second arrangement shows a 2-4 triple in II, III, IV. Therefore, although the exchange in II and III is determinable (if no overlapping occurs), the exchange in III and IV could be either a 2-strand or a 4-strand exchange. Indeterminacy of this type is confined to exchanges occurring in regions I and II or to those occurring in regions III and IV when a 2-strand double exchange simultaneously occurs in II and III. These exchanges, however, are definitely either 2- or 4-strand exchanges and can be used in calculations as to the sum of the 2- and 4-strand exchanges.

Indeterminacy Produced by a 4-strand Double Exchange Occurring in One Region

If a 2-strand double exchange occurs between two markers, it would be undetected because it would involve only the exchange of two unmarked pieces of chromosomes. A 3-strand double exchange under the same circumstances would be detected, but it would appear to be a single exchange. A 3-strand double produces one non-crossover, one double crossover, and two single crossover chromatids. Since the double crossover chromatid

would be undetected, only the two single crossover chromatids would be observed, and the entire phenomenon would register as a single crossover. However, a 4-strand double exchange occurring between two markers is detected as such, because a complete change of linkage occurs between the two markers, producing four single crossover chromatids, each one of which can be recognized. Four such exchanges were found in the 1575 asci described here. Two occurred in region I (about four units long); one in region IV (about eight units long), and one occurred in either II or III (each about 3.5 units long). This means that undetected 2-strand double exchanges probably occurred also and that some of the single exchanges recorded were actually 3-strand double exchanges.

If a 4-strand double exchange occurs in one single region, it is not possible to determine which of the four possible combinations of strands were involved. If a single exchange occurs simultaneously in another region (*e.g.*, region IV), it is not possible to classify the double exchange between regions I and IV resulting. For example ascus 6273.

This type of indeterminacy affects the analysis of the type of exchanges occurring in ascus 6283.

Two-strand Double Exchanges between Regions II and III

The conclusion reached in the present paper is that the double exchanges are distributed in non-random patterns among six pairs of regions, I–II, I–III, I–IV, II–III, II–IV, and III–IV. The types of exchanges between five of these pairs of regions are detected by recombinations of marker genes, independently of the arrangement of the spores in the ascus. Although the arrangement of the spores is used to distinguish between I–II and I–III or II–IV and III–IV exchanges, the marker genes establish the kind of double exchange (2-, 3-, or 4-strand) which has occurred, independently of the spore arrangement. The demonstration of non-randomness was established by the data obtained by the recombination technique from five pairs of these regions.

Either overlapping of the spindles or equational division of the centromere at the first meiotic division would produce an arrangement of spores

in the ascus interpreted as a 2-strand double exchange in regions II and III. Therefore, this particular type of exchange has alternative interpretations. Since the non-random patterns are established by the exchanges between the other five regions without considering this particular case, one might ask why these exchanges are considered at all. Why not simply disregard them and rely only on the conventional marker-gene technique? The answer is that if we wish to understand the pattern of exchange that occurs, the validity of these exchanges must be considered especially critically because it is only by arrangements of spores that the distribution of different types of exchanges between the two marker-genes nearest the centromere can be determined. A 2-strand double in regions II and III is undetectable except by the arrangement of the spores because the centromere produces no phenotypic effect and is a marker only insofar as is indicated by arrangement of spores in the ascus.

There is no *a priori* reason why equational division of the centromere at metaphase I or spindle overlapping should occur coincidentally with crossing over. In fact, it is a reasonable assumption that either of these improbable events occurs independently of crossing over. If the asci were reclassified and the arrangements interpreted as 2-strand double exchanges in regions II and III were re-interpreted as overlapping of the spindles, the following tabulation shows that by this reclassification, nine of the double exchanges are transferred from the double exchange to the non-crossover class and four of the quadruple exchanges are now classified as double exchanges.

TYPE OF EXCHANGE	NO. ASCI	2-STRAND DOUBLES	TRANSFERRED TO NEW CLASSIFICATION	TOTAL	CASES OF OVERLAPPING		χ^2
					OBS.	CALC.	
None	711		+9	720	9	8.67	.01
Single	330			330	0	3.97	3.97
Double	33	−9	+4	28	4	.34	39.90
Triple	2			2	0	.03	.03
Quad.	5	−4		1	0	.01	.01
							43.9

However, in this event four of the 13 cases of overlapping or equational separation of the centromere at metaphase I occur among the 28 double exchange asci. This is an extremely improbable distribution as the χ^2 test shows, and we may conclude that either the before-mentioned arrangements are not due to overlapping or equational separation of the centromere at metaphase I, or we are forced to take the highly improbable view that overlapping or equational centromere separation usually occurs coincidentally with a double exchange in regions I and IV. The former conclusion seems more reasonable than the latter. We may, therefore, conclude that overlapping or equational separation of the centromere at

anaphase I is extremely rare if either occurs at all. A corollary of this analysis is that those quadruple exchanges involving a 2-strand exchange in II and III are authenticated as quadruple exchanges. This is important because the number of quadruple exchanges is considerably in excess of expectation.

In the 1937 data, most of the 2-strand doubles are in regions I and IV and therefore are proved by gene-markers to be undisputed 2-strand double exchanges, and no supporting argument is required to establish the excess of 2-strand exchanges across the centromere. At this point it may be interesting to point out another difference between the 1937 and 1940 experiments. In the former there were ten 2-strand exchanges in I and IV and one in II and III. In the latter, there were five 2-strand exchanges in I and IV and nine in II and III. The difference is apparently significant, indicating that in the latter experiment there was a tendency for 2-strand double exchanges to occur nearer the centromere than in the earlier experiment.

Cytological Observations by Dodge in N. sitophila

The preceding argument is limited because it does not eliminate the possibility that a small fraction of the 2-strand exchanges in regions II and III may be due to overlapping. Another argument is available. DODGE (1927) in an extensive cytological investigation of the closely related eight-spored species of *N. sitophila*, did not find any case of overlapping spindles and noted a partition of vacuoles in the cytoplasm of the ascus at the four-nucleate stage effectively separating the nuclei and preventing change of position. In *N. tetrasperma*, DODGE has described the overlapping of the spindles to occur regularly in every ascus, so it is a condition with which he is very familiar. It is easy to identify because the centrosome remains attached to the nuclei after telophase and makes it possible to distinguish the orientation of the spindles even after they have disappeared.

Distribution of Double Exchanges Between the Different Regions

From table 1, it is possible to extract the different cases of double exchanges and determine the relative frequency of the different types of double exchanges in the different regions. The tabulation below contains

REGIONS	I & II		I & III		I & IV		II & III		II & IV		III & IV		II OR III	IV	TOTAL		
YEAR	37	40	37	40	37	40	37	40	37	40	37	40	40	37	37	40	37 & 40
2-strand		1		2	10	5	1	9							11	17	28
3-strand			2		5	2		2				2			7	6	13
4-strand	1	1			4	2	1			4		2	1	1	7	10	17
Total	1	2	2	2	19	9	2	11		4		4	1	1			58
2-:3-:4-	1:0:2		2:2:0		15:7:6		10:2:1		0:0:4		0:2:2						
	3		4		28		13		4		4						

only the simple double exchanges. The adjacent double exchanges may also be extracted from the triple and quadruple exchanges. Each triple exchange in regions I, II, and III produces two adjacent double exchanges, one each in I and II and one in II and III. Each quadruple exchange produces three adjacent double exchanges, one each in regions I–II, II–III, and III–IV. Non-adjacent doubles were not considered, since they were too difficult to classify and often indeterminate. This reduces the total of the double exchanges extracted from triples and quadruples.

Double exchanges extracted from triple and quadruple exchanges

REGIONS	I & II		I & III		I & IV		II & III		II & IV		III & IV		I	TOTAL		
YEAR	37	40	37	40	37	40	37	40	37	40	37	40	40	37	40	37 & 40
2-strand							6	4						6	4	10
3-strand	1	1	2					1			3	3		6	5	11
4-strand	1			1					1		2	1	2	4	4	8
2- or 4-strand	3	3									1	1		4	4	8
2- or 3- or 4-strand						1									1	1
Total	5	4	2	1		1	6	5	1		6	5	2	20	18	38

Adding the double exchanges extracted from the triples and quadruples to the simple double exchanges, the result is shown below:

REGIONS	I & II	I & III	I & IV	II & III	II & IV	III & IV	I	IV	III OR II	TOTAL
2-strand	1	2	15	20						38
3-strand	2	4	7	3		8				24
4-strand	3	1	6	1	5	5	2	1	1	25
2- or 4-strand	6					2				8
2- or 3- or 4-strand			1							1
2-:3-:4-	1:2:3	2:4:1	15:7:6	20:3:1	0:0:5	0:8:5				
	6	7	28	24	5	13				

Positive and Negative Chromatid Interference Between Different Specific Pairs of Regions

Inspection of the tabulation of simple double exchanges revealed a lack of homogeneity in the data. Therefore, the data were regrouped to determine if any homogeneous groups existed. Testing the exchanges in regions I–IV and II–III as a group revealed that they are homogeneous. As a group of exchanges, I–II and I–III were likewise homogeneous among themselves, and this was also true of the groups II–IV and III–IV. Therefore, we have three different groups (a) I–II and I–III, (b) I–IV and II–III. and (c) II–IV and III–IV.

Inspection of the adjacent exchanges extracted from the triples and quadruples reveals a similar grouping consistent with that found in the simple double exchanges. The data were rearranged as shown below:

	I–II & I–III		I–IV & II–III		II–IV & III–IV	
	OBS.	CALC.	OBS.	CALC.	OBS.	CALC.
2's	5.6	8.5	35	23.3	0	8.8
3's	6	5.0	10	13.7	8	5.3
4's	7.4	4.5	7	15.0	12	5.8

The six indeterminate 2- or 4-strand exchanges were redistributed in the I–II and I–III group according to the ratio in which the determinate exchanges had been distributed—i.e., 2's, 3/7 of 6; 4's, 4/7 of 6. The two indeterminate 2's or 4's in the II–IV and III–IV column were added to the ten 4's already classified because since no 2's were found, it seemed reasonable that these indeterminates were probably 4's. The χ^2 test shows that the three broups, a, b, and c, are significantly different from each other; $P = 4 \times 10^{-6}$. Although the data on regions I–IV and II–III are homogeneous and were therefore combined, this analysis can be made independently of the exchanges occurring between II–III. That is, if the test is made between groups (a) I–II and I–III, (b) I–IV, and (c) II–IV and III–IV, a significant lack of homogeneity will be found. In this case $P = 7 \times 10^{-4}$. Therefore, the question of the validity of the 2-strand exchanges between II and III does not change the picture of non-randomness which exists in the double exchanges between the other four pairs of regions.

The significant non-homogeneity of the groups means that exchanges in any two regions occur with a frequency distribution statistically characteristic of those particular regions. Because of the characteristic local specificity, attempts to determine the total distribution of different types of double exchanges on the basis of the sum of all the exchanges occurring along the entire chromosome are of dubious value.

The χ^2 test shows that the ratio 35:10:7 (group b, regions I–IV and II–III) is not the 1:2:1 ratio expected with no chromatid interference. $P = 1 \times 10^{-12}$. This is likewise true of the ratio 15:7:5, which includes only the exchanges in regions I and IV. $P = 6 \times 10^{-3}$. In regions I–IV as well as in regions II–III, which are symmetrically placed across the centromere, the excess of 2-strand exchanges over 4-strand exchanges (2.5:1 and 20:1 respectively) indicates that chromatid interference is negative in these regions.

The χ^2 test shows that the ratio 0:8:12 for regions II–IV and III–IV is a significant departure from a 1:2:1 ratio. $P = 3 \times 10^{-4}$. The high excesss of 4-strand exchanges shows a positive chromatid interference in these regions.

The ratio of 2's:3's:4's in regions I–II and I–III does not differ significantly from a 1:2:1 ratio.

Calculation of the Expected Distribution of Exchanges

The calculation of the numbers and types of multiple exchanges based on the number of double crossovers and analysis of chromatid data has no significance unless the assumption is made that chromatid interference does not occur. WEINSTEIN (1936) has made calculations of this type, but he pointed out that chromatid interference would affect the results. How-

ever, in tetrad analysis, the calculated number of exchange tetrads has a real significance and can be compared to a definite observed value, irrespective of the kind or degree of chromatid interference.

TABLE 3

Distribution of exchanges between chromosomes in 1081 asci.

TYPE EXCHANGES	REGIONS	NUMBER ASCI	FREQUENCY IN REGIONS			
			I	II	III	IV
None		711				
Single	I	73	73			
Singles	II	54		54		
Singles	III	61			61	
Singles	IV	142				142
2-strand						
Doubles	I–II	1	1	1		
Doubles	I–III	2	2		2	
Doubles	I–IV	5	5			5
Doubles	II–III	9		9	9	
3-strand						
Doubles	I–IV	2	2			2
Doubles	II–III	2		2	2	
Doubles	III–IV	2			2	2
4-strand						
Doubles	I–II	1	1	1		
Doubles	I–IV	2	2			2
Doubles	II–IV	4		4		4
Doubles	III–IV	2			2	2
Doubles	II or III	1		1	1	
Triple	I–III–IV	1	1		1	1
Triple	I–IV	1	2			1
Quadruple	I–II–III–IV	4	4	4	4	4
Quadruple	I–II–III	1	2	1	1	
Total		1081	95	77	85	165

We are indebted to MR. E. W. TIMM and DR. M. B. ZELLE for explaining the correct mathematical technique for calculating the expected number of multiple exchange tetrads.

Table 3 shows that a total of 95 exchanges occurred in region I, 77 in region II, 85 in region III, and 165 in region IV. Since a total of 1081 asci were analyzed, the frequency of exchanges in the four regions is 95/1081 = .0878 in region I, .0712 in region II, .0787 in region III, and .1525 in

region IV. If these values are called p, the frequency of exchange, then $1-p$ is the frequency of no exchange. The following tabulation shows the method which is based on the assumption of no chromosome interference.

EXCHANGE	
None	$.9122 \times .9288 \times .9213 \times .8475 \times 1081 = 714.0$
Single I	$.0878 \times .9288 \times .9213 \times .8475 \times 1081 = 68.8$

The observed and calculated values of the different categories appear below.

	1940			1937		
EXCHANGE	OBS.	CALC.	χ^2	OBS.	CALC.	χ^2
None	711	714	0	339	319	1.3
S I	73	68.8	.3	24	39.1	5.8
S II	54	54.8	.0	18	19.2	.1
S III	61	61.1	.0	16	19.2	.5
S IV	142	128.8	1.3	63	72.2	1.2
D I–II	2	5.3	2.1	1	2.5	.9
D I–III	2	5.9	2.6	2	2.5	.1
D I–IV	9	12.4	.9	19	8.8	11.8
D II–III	11	7.1	2.1	2	1.2	.5
D II–IV	4	9.9	3.5	0	4.3	4.3
D III–IV	4	11.0	4.5	0	4.3	4.3
T I–II–III		.45	.45	2	.14	24.6
T I–II–IV		.95	.95	1	.53	.4
T I–III–IV	1	1.05	.0	2	.53	4.1
T II–III–IV		.84	.84	2	.26	11.7
Q I–II–III–IV	4	.08	192.00	2	.03	129.0

Anomalous multiple exchanges could not be classified to fit this table. They are (1) the 4-strand double in regions II or III, (2) the triple in I–IV, and (3) the quadruple in I–II–III. Therefore, the total of multiple exchanges is even larger than the table indicated. The value of χ^2 for $P = .01$ is 24.7 when $n = 11$. The exceedingly high value of χ^2 shows that five quadruple exchanges are an unusually large excess over those expected.

A highly significant excess of quadruple exchanges was found, indicating that the widely held view that multiple exchanges are usually less frequent than expected does not apply in Neurospora.

Local Patterns of Negative and Positive Chromosome Interference

In studying chromatid interference, the types of double exchanges occurring in certain regions were found to be homogeneous so the data were combined. The same combinations are made again in the following tabulation to test for chromosome interference among the different regions. Only the simple double exchanges are used, for the best expression of interference should be apparent in comparing the observed and calculated values of the simple double exchanges.

REGIONS	OBS.	CALC.	TETRAD COINCIDENCE*	RATIO 2's:4's† (A) DOUBLE EXCHANGES	(B) COMBINED EXCHANGES
(a) I–II & I–III	7	16.2	.43	1.5	.75
(b) I–IV & II–III	41	29.5	1.39	3.6	5.0
(c) II–IV & III–IV	8	29.5	.27	0.0	0.0

* measures chromosome interference
† measures chromatid interference

The ratio of the observed number of double exchanges to the calculated number of doubles has been called "tetrad coincidence." This is a better measure of chromosome interference than standard coincidence because it is independent of any assumption concerning the frequency of the different types of double exchanges.

The last two columns show the ratio of 2-strand to 4-strand exchanges. The first column (a) is compiled from only the simple double exchanges. The second column (b) contains in addition the 2-strand and 4-strand exchanges extracted from the triple and quadruple exchanges as well as the simple double exchanges. The ratio gives the measure of the degree of chromatid interference. If it is larger than 1, there is an excess of 2-strand exchanges and therefore chromatid interference is negative; if it is less than 1, there is an excess of 4-strand exchanges and therefore chromatid interference is positive. When chromatid interference does not exist, this ratio will be equal to 1.

Chromosome interference is least between regions showing the largest excess of 2-strand exchanges and, therefore, the least degree of chromatid interference. When chromosome interference is highest, the 4-strand exchanges are in excess, and therefore chromatid interference is also at a maximum.

An excess of multiple exchanges does not necessarily mean the complete absence of chromosome interference, and the old concept of chromosome interference may require considerable modification. Tetrad analysis shows that more variables are operating than was previously thought to be the case and that methods of calculation based on chromatid analysis cannot predict the expected numbers and types of exchanges.

Recalculation of the 1939 Data

Mr. Everett R. Dempster pointed out that failure to recognize 2- and 4-strand indeterminacy led to an error in the calculations in the 1939 paper. In these experiments zygotes heterozygous for peach, tuft, and fluffy (linked genes in the second chromosome of *Neurospora crassa*) were analyzed. The following are the recalculated data:

REGIONS	2-STRAND	3-STRAND	4-STRAND	INDETERMINATE 2- OR 4-STRAND
Double I–II		6		5
Double I–III		6		9
Double II–III	2	3	2	
Double III			2	
Triple I–II–III	3	2	—	1
	5	17	4	15

These data are homogeneous for the different combinations of regions and, considering the indeterminacy of 2's and 4's, do not differ from randomness. The total of the 3-strand exchanges (17) does not differ significantly from the sum of all the 2's and 4's (24).

Calculation of the expected numbers of different types of exchanges gives the following result:

	OBSERVED	CALCULATED	TETRAD COINCIDENCE
No Exchanges	104	110.7	
Single I	34	32.4	
Single II	26	22.6	
Single III	76	67.5	
Double I–II	11	6.6	1.66
Double I–III	15	19.7	.76
Double II–III	9	13.7	.51
Double II	2		
Triple I–II–III	3	4.0	

DISCUSSION

EMERSON and BEADLE (1933) and BEADLE and EMERSON (1935) concluded that their Drosophila data indicated that crossing over occurred at random, without the occurrence of chromatid interference. They might have reached a different conclusion if (1) they had used shorter distances and (2) had not combined the different kinds of exchanges found along the entire chromosome without respect to the homogeneity of the data.

The regions on the sex chromosome of Neurospora mapped for this analysis are respectively about 4, 3.5, 3.5, and 8 units long. Our data show that the last region was much too long and better results would have been obtained if a marker gene had been located between crisp and pale. This would have been especially valuable since it would have balanced the regions symmetrically placed across the centromere. Moreover, the occurrence of 4-strand double exchanges in single regions shows that even the short regions were not short enough. Four of these 4-strand exchanges were detected; two in region I, one in region II or III; and one in region IV. Since this is the only type of double exchange detectable in a single region, undetected 2- and 3-strand exchanges probably occurred in all regions.

The distances used by BEADLE and EMERSON were 3.5, 5.8, 13.7, 10.0, 13.0, 6.3, 8.2, and 5.5, respectively. Most of these regions are far too long, and naturally most of their crossovers occurred between the longer regions. Only three regions are short enough to yield analyzable results, and two

TABLE 4

Distribution of exchanges between chromosomes in 494 asci (1937).

TYPE EXCHANGES	REGIONS	NO. ASCI	FREQUENCY IN REGIONS			
			I	II	III	IV
None		339				
Single	I	24	24			
Single	II	18		18		
Single	III	16			16	
Single	IV	63				63
2-strand						
Double	I–IV	10	10			10
Double	II–III	1		1	1	
3-strand						
Double	I–III	2	2		2	
Double	I–IV	5	5			5
Double	IV	1				2
4-strand						
Double	I–II	1	1	1		
Double	I–IV	4	4			4
Double	II–III	1		1	1	
Triple	I–II–III	1	1	1	1	
Triple	I–II–IV	1	1	1		1
Triple	I–II–III	1	1	1	1	
Triple	I–III–IV	2	2		2	2
Triple	II–III–IV	1		1	1	1
Triple	II–III–IV	1		1	1	1
Quadruple	I–II–III–IV	2	2	2	2	2
		494	53	28	28	91

of these are separated from the third by five very long regions. The additional fact that BEADLE and EMERSON combined all their data to get statistically significant results obscured the existence of any local patterns which might have been present.

BONNIER and NORDENSKIÖLD (1937) published conclusions contrary to those of EMERSON and BEADLE. BONNIER and NORDENSKIÖLD found an

excess of 4-strand exchanges and attributed the difference between their results and those of EMERSON and BEADLE to the fact that they (BONNIER and NORDENSKIÖLD) had selected parents which resulted in a higher survival of critical progeny.

SUMMARY

Tetrad analysis of crossing over between four regions on the sex chromosome of *Neurospora crassa* (I and II on the left of the centromere and III and IV on the right) revealed locally specific patterns of chromatid and chromosome interference as follows:

Between regions I and IV and similarly between regions II and III, there was a high degree of negative chromatid interference and a high degree of negative chromosome interference. This produced more than the expected amount of crossing over and an excessive number of 2-strand exchanges. These regions are symmetrically placed across the centromere.

Between regions I and II, and similarly between regions I and III, there was no evidence of chromatid interference. However, there was a significantly high degree of positive chromosome interference.

Between regions II and IV, and similarly between regions III and IV, there was a high degree of positive chromatid interference and a high degree of positive chromosome interference yielding few double exchanges which were preponderantly of the 4-strand type.

In view of this demonstration of local distribution of specifically different crossover patterns, it is manifestly impossible to draw any conclusions concerning the randomness of crossing over by indiscriminately combining data collected from different regions along the chromosome.

An excessively high proportion of triple and quadruple exchanges was found. At the same time, between certain regions positive chromosome interference was demonstrated. Therefore, a limited amount of positive chromosome interference does not necessarily exclude the possibility of an excessive number of multiple exchanges occurring.

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MEIOTIC CHROMOSOME BEHAVIOR IN SPECIES, SPECIES HYBRIDS, HAPLOIDS, AND INDUCED POLYPLOIDS OF GOSSYPIUM[1]

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INTRODUCTION

THE genus Gossypium, a member of the Malvaceae, includes the species of cultivated cottons. More than 100 species of Gossypium have been described, but recent workers include all the described types in fewer than 20 species (HUTCHINSON and GHOSE 1937; HARLAND 1939). The basic chromosome number in the genus is 13, and all species studied have either 13 or 26 pairs of chromosomes.

The cytological behavior of all species hybrids, except one, included in this paper has been reported previously by other workers. Further data on the meiotic chromosome behavior of species hybrids and haploids are given, together with a review of published data. Data on the meiotic chromosome behavior of several induced polyploids of Gossypium are given for the first time. In the present work emphasis is placed upon data relative to the amount of structural differences among chromosomes of different species and to the meiotic chromosome behavior of polyploids. These data are used in an attempt to define more accurately the relationships of Gossypium species and to make clearer the process of speciation of the genus.

GENERAL CONSIDERATIONS

The more comprehensive previous works on the meiotic chromosome behavior of Gossypium hybrids were published by SKOVSTED (1933, 1934, 1935a, 1937) and WEBBER (1935, 1939). These two authors adequately review the earlier papers on the subject. The data published by SKOVSTED, WEBBER, BARDUCCI and MADOO (1940), and ABRAHAM (1940) are summarized in table 1. In making the summary, it seemed desirable in some instances to combine data from two or more similar crosses. Some of the published cytological data which are unrelated to this study are omitted. In studying hybrids, SKOVSTED and WEBBER recorded the number of univalents, bivalents, trivalents, etc., and SKOVSTED recorded chiasma frequency. In some cases the data published independently by SKOVSTED and by WEBBER from the same or similar hybrids are greatly different.

[1] This work was initiated under the direction of the late PROF. E. M. EAST and submitted to HARVARD UNIVERSITY as part of a thesis in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Much of the cytological work was done while the author was Agent, U. S. DEPARTMENT OF AGRICULTURE, Bureau of Plant Industry, Raleigh, N. C.

SKOVSTED (1937) separated Gossypium species into three, WEBBER (1939) separated them into four, and HARLAND (1939) separated them into six general types. These authors considered morphology and chromosome number and gave some attention to chromosome pairing in hybrids and to geographical distribution. In the present work, with more emphasis placed on chromosome pairing and structural differences among the chromosomes, the species are divided into six types. One of HARLAND'S types is divided, while two others are combined.

In table 2 and throughout this paper formulae are used to describe the chromosome composition of species and hybrids. These formulae are written by using different capital letters to designate structurally unlike sets of chromosomes and by placing numbers before the letters to indicate the number of times sets are present (BEASLEY 1940a). Closely related sets are distinguished by placing subscripts 1, 2, etc., after the letters that designate the sets. Sets in natural polyploids are enclosed with parentheses with subscripts after the last parenthesis. The evidence for the relationships indicated among the sets of chromosomes in table 2 is given later in this paper, but the formulae are used throughout because they facilitate the description and designation of plants.

Since emphasis in this paper is placed on structural differences among chromosomes of different species, particular attention is given to chromosome bridges at first meiotic anaphase. Such chromosome bridges have been shown in many plants to result from crossing over in individuals heterozygous for inversions or inverted translocations which gives chromosomes with two centromeres. The bridges are formed when the centromeres go to opposite poles.

The concept that chromosome bridges result from differences in chromosome structure was advanced by MCCLINTOCK (1933). She found that crossing over sometimes occurs in heterozygous inversions in which homologous parts pair by forming a loop. This crossing over gives dicentric chromosomes which frequently form bridges at anaphase. MÜNTZING (1934) pointed out that chromosome bridges in species hybrids are the result of structural differences in chromosomes. His diagrams I to IV illustrate how bridges can arise from certain types of translocations. RICHARDSON (1936) diagrammed several ways in which bridges may arise in structural hybrids. SAX (1937) reported chromosome bridges in Paeonia to result from crossing over in heterozygous inversions. He found inversions in three of the five chromosomes and reported that heterozygous inversions caused partial asynapsis. Numerous other workers, including DARLINGTON (1936, 1937), DOBZHANSKY (1937), UPCOTT (1937), WOODS (1937), EMSWELLER and JONES (1938), STEBBINS and ELLERTON (1939), and SWANSON (1940), and several others, have studied structural differences

in chromosomes. Species with large chromosomes were usually used. The number of structural differences was one to five, and bridges could be interpreted readily as resulting from heterozygous inversions or inverted translocations.

In the present work some first meiotic anaphase figures of species hybrids were found to have a larger number of bridges than is usually reported. It is believed that comparable numbers have been observed in other genera. It has been suggested that the large number of attenuated chromosomes in certain hybrids could be a physiological effect resulting from the hybridization of the species. This is untrue in Gossypium, for the chromosomes fail to become attenuated in polyploids produced from the hybrids. The drawings of meiotic figures of Nicotiana hybrids published by GOODSPEED (1934) indicate that types with a reduced and variable amount of pairing frequently give chromosome bridges at anaphase.

In the work with Gossypium, or other species with small chromosomes, it is hardly possible to make the detailed observations that can be made on material with large chromosomes. Perhaps one reason figures interpreted in the present work as bridges were not so interpreted by previous workers is that the number of fragments is usually less than the number of bridges. It is possible, however, that the fragments are rarely freed at anaphase. MCCLINTOCK (1938) found that in about one-third of her observations a fragment was "associated at one of its ends with the end of one of the normal chromatids." Probably another reason why the number of fragments is lower than the number of bridges is that in many cases the bridges are not the result of crossing over in inversions, but the result of changes in homology which prevent terminalization (DARLINGTON 1937). The point emphasized in this paper is that bridges, whether they arise from crossing over in inversions or by other means, result from structural differences among the chromosomes of different species. Structural differences are interpreted here to include segments with genes that may not be in one of the two species in a hybrid—that is, non-homologous segments.

It is customary for conclusions as to relationships between species to be based largely upon the amount of chromosome pairing in hybrids between the species; it is known, however, that changes in chromosome structure, which influence chromosome pairing, apparently can occur without accompanying genetic effect (ANDERSON 1935; STEBBINS 1938). In Gossypium it is probable that the amount of chromosome pairing is a good measure of relationship, for, in general, it agrees with geographical distribution and external morphology.

TECHNIQUE

Flower buds were killed in a mixture of 30 parts acetic acid and 70 parts

TABLE 1

*Meiotic chromosome behavior in species hybrids of Gossypium.**

GROUP	TYPES CROSSED	SPECIES CROSSED†	BEHAVIOR OF CHROMOSOMES AT M I AND A I								AUTHOR
			I‡	II	III	IV	V	VI	CHIASMA FREQUENCY PER BIVALENT	EVIDENCE OF STRUCTURAL DIF. IN CHROMOSOMES	
1	2A×2A (Asiatic 13×Asiatic 13)	*sanguineum*×*africanum*	sometimes 2	13.0							WEBBER 1935
		herbaceum×*cernuum*	0.2	12.9							WEBBER 1939
		herbaceum×*arboreum*	sometimes 2	13.0					reduced from normal		SKOVSTED 1933
2	2A×2B (Asiatic 13×African 13)	*arboreum*×*anomalum*	3.1	10.5	0.2	0.4			1.37	Text fig. 3 gives evidence of A I bridges	SKOVSTED 1937
		arboreum var. *nanking*×*anomalum*	1.7	11.7	0.2	0.1			1.48		SKOVSTED 1937
		herbaceum var. *africanum*×*anomalum*	2.5	11.8					1.41	Text fig. 4 gives evidence of A I bridges	SKOVSTED 1937
		sanguineum×*anomalum*	2.4	11.8							WEBBER 1939
3	2A×2C (Asiatic 13×Australian 13)	*arboreum*×*sturtii*	2.8	9.8	0.6	0.4			1.32	Text figs. 7 & 8 give evidence of A I bridges	SKOVSTED 1937
		herbaceum×*sturtii*	21.6	2.2							WEBBER 1939
4	2A×2D (Asiatic 13×American 13)	*arboreum*×*thurberi*	9.6	7.8	0.2	0.05			1.16	Text figs. 12–15 show bridges	SKOVSTED 1937
		nanking×*thurberi*	24.0	1.0							WEBBER 1939
5	2A×2E (Asiatic 13×Arabia-India 13)	*arboreum*×*stocksii*	12.0	7.0							ABRAHAM 1940
		herbaceum var. *wightiana*×*stocksii*	19.6	3.2					1.05		SKOVSTED 1937
		arboreum var. *nanking*×*stocksii*	17.3	4.4					1.07		SKOVSTED 1937
		arboreum×*stocksii*	13.5	6.2					1.11	Text fig. 6 shows bridges	SKOVSTED 1937
		sanguineum×*stocksii*	24.1	0.9							WEBBER 1939
6	2A×2(AD) (Asiatic 13×American 26)	*arboreum*×*barbadense*	14.4	8.4	1.0	0.6	0.3	0.1		Text figs. 6–8 show evidence of bridges	SKOVSTED 1934
		asiatic×*hirsutum*	13.7	11.9		0.35					WEBBER 1935
		cernuum×*hirsutum*	13.2	12.7	0.1					Rarely nondisjunction occurred	WEBBER 1939
7	2B×2D (African 13×American 13)	*anomalum*×*aridum*	21.2	2.4	0.05				1.03		SKOVSTED 1937
		anomalum×*thurberi*	14.3	5.7	0.01				1.12	Text fig. 11 shows bridges	SKOVSTED 1937
		anomalum×*davidsonii*	19.9	3.1					1.08	Text fig. 10 shows 1 bridge	SKOVSTED 1937
		anomalum×*davidsonii*	25.6	0.2							WEBBER 1939
8	2B×2(AD) (African 13×American 26)	*anomalum*×*barbadense*	33.8	2.6					1.05	Text fig. 22 shows 4 bridges	SKOVSTED 1937
		anomalum×(*barbadense*×*schottii*)	18.1	10.3	0.1						WEBBER 1939
		anomalum×(*contextum*×*hopi*)	17.8	10.5	0.1						WEBBER 1939
9	2C×2D (Australian 13×American 13)	*sturtii*×*harknessii*	24.6	0.7						Reports fragmenting chromosomes	WEBBER 1935
		sturtii×*thurberi*	24.5	0.7						Fig. 11 shows 5 bridges	WEBBER 1935, 1939
		sturtii×*armourianum*	24.1	1.0						Reports fragmenting chromosomes	WEBBER 1935

		sturtii×*armourianum*	8.5	8.2	0.3	0.1			Skovsted 1937
		sturtii×*davidsonii*	14.9	5.3	0.1	0.03	1.06	Text figs. 6–8 show several bridges	Skovsted 1935
		sturtii×*davidsonii*	24.0	1.0				Reports fragmenting chromosomes	Webber 1935
10	2C×2(AD) (Australian 13×American 26)	*sturtii*×*barbadense*	37.2	0.9				Reports fragmenting chromosomes	Webber 1935
		sturtii×*barbadense*	32.5	3.1	0.1		1.0	Text fig. 25 shows 5 bridges	Skovsted 1937
		sturtii×*hirsutum*	21.0	7.6	1.0				Skovsted 1937
		sturtii×*hirsutum*	37.9	0.5					Webber 1930
		sturtii×*purpurascens*	24.3	6.0	0.8	0.05	1.05	Text fig. 26 shows 8–10 bridges	Skovsted 1937
		sturtii×*darwinii*	27.1	5.3	0.4	0.05	1.02	Text fig. 27 shows 4–6 bridges	Skovsted 1937
11	2D×2D (American 13×American 13)	*thurberi*×*aridum*	sometimes 2	13.0			1.73		Skovsted 1937
		amourinaum×*thurberi*	sometimes 2	13.0			1.57	Text fig. 2 shows 1 probable bridge	Skovsted 1937
		amourianum×*thurberi*	0.6	12.7					Webber 1930
		armourianum×*aridum*		13.0					Skovsted 1937
		davidsonii×*klotzschianum*	0.1	12.95					Webber 1930
		harknessii×*armourianum*		13.0					Webber 1935
		harknessii×*thurberi*	0.8	12.6					Webber 1930
12	2D×2AD (American 13×American 26)	*armourianum*×*hirsutum*	13.0	13.0					Webber 1934, 1935
		armourianum×*hirsutum* (Vy)	13.5	12.2	0.4				Skovsted 1937
		armourianum×*hirsutum* (V4)	11.8	13.4	0.2				Skovsted 1937
		thurberi×*hirsutum*	13.1	12.7	0.2				Webber 1930
		thurberi×*hirsutum*	13.5	12.5	0.2		1.54		Skovsted 1937
		harknessii×*barbadense*	13.0	13.0					Webber 1935
		armourianum×*contextum*	13.0	12.7		0.1			Webber 1935
		aridum×*barbadense*	14.0	12.3	0.2		1.73	Text fig. 16 shows possible bridges	Skovsted 1937
		aridum×*hirsutum*	13.2	12.4	0.4			Text fig. 17 shows 1 bridge	Skovsted 1937
		aridum×*purpurascens*	13.0	12.0	0.6	0.05	1.64		Skovsted 1937
		armourianum×*purpurascens*	13.8	12.4	0.04		1.49	Text fig. 19 shows possible bridges	Skovsted 1937
		armourianum×*latilense*	14.5	12.1	0.1		1.55		Skovsted 1937
		armourianum×*barbadense*	14.8	12.0	0.1				Skovsted 1937
		armourianum×*darwinii*	13.2	12.0	0.6				Skovstfd 1937
		thurberi×*barbadense*	12.7	12.4	0.5			Text fig. 21 shows 2 or more bridges	Skovsted 1937
		thurberi×*latilense*	13.4	12.1	0.5				Skovsted 1937
		hirsutum×*raimondii*	12.6	11.6	0.9	0.1			Barducci and Madoo 1940
13	2E×2(AD) (Arabia-India 13×American 26)	*barbadense*×*stocksii*	37.9	0.6			1.00		Skovsted 1937

* Although the species names given by the authors fail in some cases to conform to the classification given by Hutchinson and Ghose (1937) and Harland (1939), the names have been retained here, for without specimens it is hardly possible to change the names to follow the recent classifications.
† In these species crosses the first name does not necessarily indicate that it was used as female.
‡ I—univalent, II—bivalent, etc.

TABLE 2

Types of Gossypium species as determined by their geographical distribution, morphology, chromosome pairing, chromosome number, and structural differences among sets of chromosomes.

	CHROMOSOME CONSTITUTION
Asiatic 13-chromosome	
G. herbaceum L.	$2A_1$
G. arboreum L.	$2A_2$
African 13-chromosome	
G. anomalum Wawra. and Peyr.	$2B_1$
Australian 13-chromosome	
G. sturtii F. Muell.	$2C_1$
American 13-chromosome	
G. thurberi Tod.	$2D_1$
G. armourianum Kearney	$2D_2$
G. harknessii T. S. Brandeg.	$2D_3$
G. davidsonii Kellogg	$2D_3$
G. klotzschianum Anderss.	$2D_3$
G. aridum (Rose and Standley) Skovsted	$2D_4$
G. raimondii	$2D_5$
Arabia-India 13-chromosome	
G. stocksii M. Mast	$2E_1$
American 26-chromosome	
G. hirsutum L.	$2(AD)_1$
G. barbadense L.	$2(AD)_2$

of either 95 percent or absolute ethyl alcohol. It was found that under average growing conditions, meiotic metaphase figures were common in material collected from 7 to 8 a.m., and anaphase figures were frequent in material collected one hour later. Unusually warm nights followed by sunny days advance the stages somewhat, while cool, cloudy weather retards the stages. Better slides were obtained after buds were left in acetic-alcohol at least three days. The stain used was BELLING'S (1926) iron-acetocarmine. Most slides were sealed with a mixture of equal parts of gum mastic and paraffin; however, slides sealed in this manner usually spoiled after a few weeks. It was found that a self-sealing aceto-carmine mixture recommended by ZIRKLE (1937) caused too much plasmylosis. Some unsealed slides were made permanent by removing the cover glasses in 70 percent alcohol and taking the slides and cover glasses through the remainder of the alcohol series to xylol and then mounting in balsam. In making sealed slides permanent, the method of MCCLINTOCK (1929) was followed.

CHROMOSOME BEHAVIOR IN PURE SPECIES

SKOVSTED (1933) found no abnormalities at first meiotic metaphase (M I) in PMC of *Gossypium arboreum*, $2A_1$, a 13-chromosome cultivated Asiatic cotton. In a triploid Asiatic cotton, however, he found that most

of the M I plates showed one or more associations of more than three chromosomes. In a later paper (1935a), he reported autosyndesis in both sets of chromosomes in a cross between two distantly related 13-chromosome species. WEBBER (1934) reported 11 percent of first anaphase (A I) figures of an Asiatic 13-chromosome species to have lagging bivalents, and 9.5 percent of second meiotic metaphase (M II) plates to have less than the haploid number of chromosomes. In the present work, although the studies made were not extensive, no abnormalities were found in normal 13-chromosome species. SKOVSTED (1933, 1937) reported chiasma frequency in Gossypium to average about 1.75 per bivalent in pure species.

A normal M I of a PMC of *G. arboreum* var. *neglectum*, $2A_2$, is shown in Plate 1, figure A, and one of *G. hirsutum*, $2(AD)_1$, is shown in Plate 1, figure B. Sharp disjunction of the chromosomes, a characteristic of pure Gossypium species, may be seen in the A I figure of *G. hirsutum* shown in Plate 1, figure C.

Meiosis in 26-chromosome Gossypium species frequently shows abnormalities. WEBBER (1934) reported 7.6 percent of M I figures to have associations of four chromosomes, and later (1938) found 14 percent of the pollen mother cells at M I to have either univalents, associations of four or both. The maximum number of univalents or of associations of four chromosomes found in a single cell was two. In the present work normal 26-chromosome species were studied to a limited extent. Bridges, lagging chromosomes, and groups of four chromosomes occasionally were found.

CHROMOSOME BEHAVIOR IN AUTOPOLYPLOIDS

In M I of 14 PMC of a $4A_1$ plant from *G. herbaceum* the number of quadrivalents ranged from 7 to 12 and averaged almost 10. In some cases it was difficult to determine whether there were two or four chromosomes in a group, but the total number of chromosome groups was counted and the number of quadrivalents calculated. Each of two cells had one univalent and one trivalent. A count of 25 A I figures gave 11 that separated 26-26, eight that separated 25-27, two that separated 24-28, one that separated 23-29, and three had chromosomes in the cytoplasm that probably would not have reached either pole.

Similar results at A I were secured in tetraploid Datura (BELLING and BLAKESLEE 1924) and in tetraploid *Lycopersicum esculentum* (Upcott 1935). JØRGENSEN (1928) and HUMPHREY (1934) reported abnormalities to be rare at A I of tetraploid *L. esculentum*.

In order to calculate the number of A I figures that had balanced 26 chromosome sets, it is necessary to subtract two, the number of 24-28 separations that were found, from the 26-26 separations, since if two quadrivalents separate 1-3, there is, so far as is known, an even chance

that the unbalance will be in opposite directions, which would give a count of 26-26 at A I. This makes it necessary to double the number in the 24-28 class (BELLING and BLAKESLEE 1924). These corrections indicate that nine of the 25 separations should have been balanced 26-26 distributions, and the plant should have given 36 percent of viable pollen. Some flowers have as much as 30 percent of apparently viable pollen.

From the data on the $4A_1$ Gossypium it was calculated that quadrivalents separate in a 3-1 manner about 13 percent of the time (22 cells averaged ten quadrivalents each, 28 quadrivalents separated 3-1). If it is assumed that there is an even chance of quadrivalents separating 2-2 or 1-3, the chance of ten quadrivalents in the same cell giving a balanced type would be $(\frac{1}{2})^{10}$ or 1/1024. The chance of the separations being something other than 26-26 would be about 4 to 1. Considering only the cells in which all the chromosomes were going to the poles, the 26-26 separations in the $4A_1$ Gossypium amounted to 50 per cent. It appears that quadrivalents separate 3-1 more than 13 percent and less than 50 percent of the time. BELLING and BLAKESLEE (1924) calculated that quadrivalents in tetraploids of Datura separate 3-1 about three percent of the time.

It was observed that bridges are sometimes present at A I in $4A_1$ plants. The most logical explanation is that some chromosomes within the basic A set have segments in common, and if segments fail to pair with like segments in homologous chromosomes, they will sometimes pair with like

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EXPLANATION OF PLATE I

FIGURE A.—First meiotic metaphase of an Asiatic 13-chromosome species *G. arboreum* var. *neglectum*.

FIGURE B.—First meiotic metaphase of an American 26-chromosome species *G. hirsutum*.

FIGURE C.—First meiotic anaphase of American 26-chromosome species *G. hirsutum* showing sharp separation of chromosomes.

FIGURE D.—First meiotic anaphase of haploid, $(AD)_1$, plant of *G. barbadense* showing 26 chromosomes.

FIGURE E.—First meiotic metaphase of octoploid $2[(AD)_1(AD)_2]$ from *G. hirsutum* and *G. barbadense*. Most of the chromosomes are paired as quadrivalents.

FIGURE F.—First metaphase of American 26- ×American 13-chromosome species, *G. hirsutum* ×*G. harknessii*, $(AD)_1D_2$, showing 12 associations of two chromosomes and 15 univalents.

FIGURE G.—First meiotic metaphase showing most of the chromosomes paired in a hybrid $A_2D_1(AD)_1$ produced by crossing an amphidiploid synthesized from an Asiatic 13- and an American 13-chromosome species with an American 26-chromosome species (*G. arboreum*×*G. thurberi*, amphidiploid)×*G. hirsutum*.

FIGURE H.—First meiotic anaphase of American 26- ×Australian 13-chromosome species, *G. hirsutum*×*G. sturtii*, $(AD)_1C_1$, showing eight bridges.

FIGURE I.—First meiotic anaphase of hexaploid $2[(AD)_1C_1]$ showing most of the chromosomes separating without forming bridges.

FIGURE J.—First meiotic anaphase of American 26- ×African 13-chromosome, *G. barbadense* ×*G. anomalum* $(AD)_2B_1$ showing nine or ten bridges.

FIGURE K.—First meiotic metaphase with 39 bivalents of a hexaploid from American 26- ×African 13-chromosome $2[(AD)_1B_1]$.

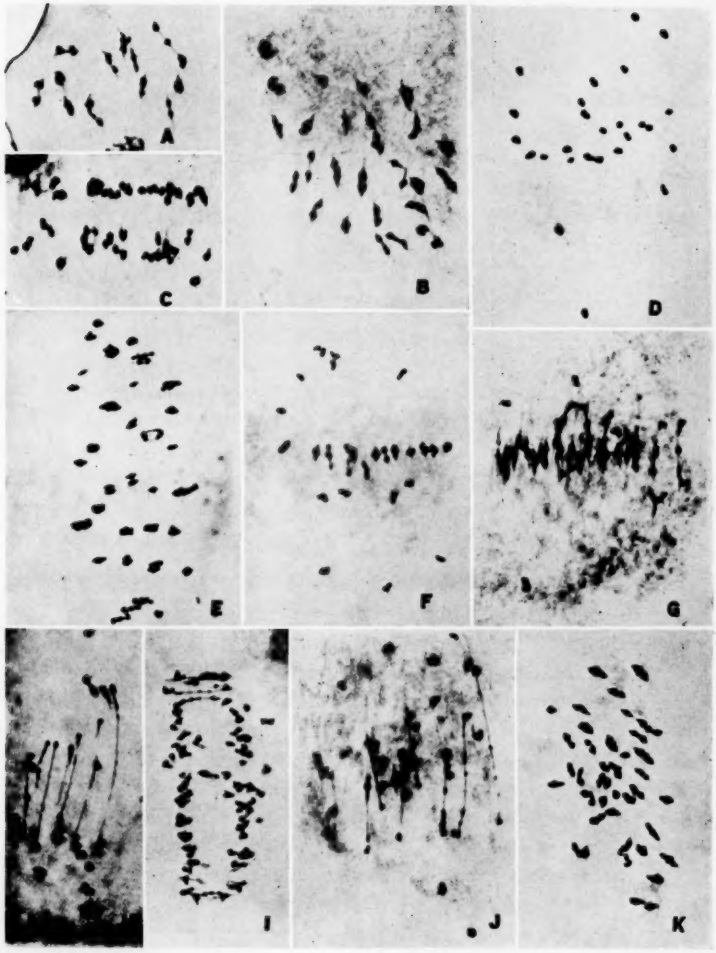

PLATE 1

segments in otherwise non-homologous chromosomes. The autosyndesis reported by SKOVSTED (1935a) and the presence of associations of more than three chromosomes in a triploid (SKOVSTED 1933) also indicate that this is true.

Doubling the chromosome number in *G. hirsutum*, $2(AD)_1$, and *G. barbadense*, $2(AD)_2$, gave $4(AD)_1$ and $4(AD)_2$ plants. At M I of these plants it usually was impossible to determine definitely whether a few associations of chromosomes were bivalents or quadrivalents (Plate 1, fig. E). It was possible, however, to count the number of chromosome bodies in some M I figures, and univalents could be identified. With the number of univalents identified and the total number of chromosome associations of two or more known, the number of quadrivalents was calculated. It was assumed that there was a trivalent for each univalent, and this was found to be true in a number of figures.

At M I 33 cells of a $4(AD)_1$ plant averaged 17.5 quadrivalents, 0.4 trivalents, 16.2 bivalents, and 0.4 univalents, while 18 cells of a $4(AD)_2$ plant averaged 18.1 quadrivalents, 0.8 trivalents, 14.2 bivalents, and 0.8 univalents. MENDES (1940) reported most of the A I and A II figures of an octoploid of *G. hirsutum* to have 52 chromosomes at each pole. He gave no data on M I. The high percentage of quadrivalents and the frequent presence of univalents and trivalents found in octoploids in the present work indicate that most of the microspores and megaspores of such plants would be unbalanced.

CHROMOSOME BEHAVIOR IN HAPLOIDS

Haploid, (AD), plants from American tetraploid species of Gossypium were reported by SKOVSTED (1933) to have a small number of chromosome associations at M I. WEBBER (1938) reported an average of 0.2 associations of two chromosomes and occasional fragmenting chromosomes. In the present work, both $(AD)_2$ (haploids of *G. barbadense*) and $(AD)_1$ (haploids of *G. hirsutum*) were studied cytologically. The interpretations of figures at M I and A I of $(AD)_2$ plants were usually subject to doubt, for frequently two chromosomes were together, as shown in Plate 1, figure D, and it was impossible to tell whether they were joined by chiasmata. If figures of that type are considered as associations of two chromosomes, an average of 0.8 bivalents was found at M I in $(AD)_2$ plants.

More conclusive data were secured from the A I figures of $(AD)_1$ plants of *G. hirsutum*, since associations of two chromosomes usually give bridges at A I (fig. 1). The average number of chromosome bridges at A I in 66 cells was 1.7, and the maximum number was five. The difference in the number of chromosome associations in $(AD)_1$ and $(AD)_2$ plants is probably genic, rather than chromosomal, for there are slight, if any, structural differences evident in crosses between $2(AD)_1$ and $2(AD)_2$ species.

FIGURE 1.—First meiotic anaphase of haploid $(AD)_1$ plant of *G. hirsutum* showing four bridges.

FIGURE 2.—First meiotic metaphase of hexaploid $2[(AD)_1A_1]$ from American 26- × Asiatic 13-chromosome species, *G. hirsutum*×*G. herbaceum*, showing univalents and multivalents.

FIGURE 3.—First meiotic anaphase of hexaploid $2[(AD)_1A_1]$ showing an extra chromosome (fragment?) at each pole.

FIGURE 4.—First meiotic anaphase of hexaploid $2[(AD)_1A_1]$ showing aberrant chromosome behavior.

FIGURE 5.—First meiotic metaphase of tetraploid $2A_1(AD)_1$ from *G. herbaceum* autotetraploid ×*G. hirsutum* showing the majority of the 3A sets associated as trivalents.

Associations of two chromosomes are common in haploids derived from polyploids, and in some cases they are present in haploids from diploids (DARLINGTON 1937, IVANOV 1938). An average of 1.7 and a maximum of five chromosome bridges at A I of $(AD)_1$ plants show that there is considerable duplication of chromosome segments in 2(AD) species. In fact, $(AD)_1$ plants behave cytologically much like the hybrid B_1D_4 of *G. anomalum*×*G. aridum* (table 1, group 7).

CHROMOSOME BEHAVIOR IN HYBRIDS AND POLYPLOIDS OF CLOSELY RELATED SPECIES

The hybrid A_2A_1 of *G. arboreum* var. *typicum*×*G. herbaceum* var. *africanum* frequently showed a bridge and fragment at A I or A II, and figures were found with one association of four chromosomes. Sometimes there was evidence of two bridges, and one A I was found with an extra chromosome going to one pole. Figures that appeared normal were common. SKOVSTED (1933) reported that a cross of *G. herbaceum*×*G. arboreum* had a lower chiasma frequency than the pure species and that sometimes the chromosomes separated 12-14 at A I. From a study of similar crosses, WEBBER (1935) reported an occasional lagging bivalent and some M II plates with 12 and 14 chromosomes. The presence of a bridge accompanied by a fragment and M I figures showing one association of four chromosomes is good evidence that the chromosomes of these two species differ by at least one translocation. WEBBER (1935) found no irregularities in a cross of two $2D_2$ species, *G. harknessii*×*G. armourianum*. SKOVSTED (1937) reported very few irregularities in three crosses between American 13-chromosome species. The cross D_2D_1, *G. armourianum*×*G. thurberi*, had a reduced chiasma frequency, and SKOVSTED's text figure 2 shows a probable bridge. One cell in his cross *G. thurberi*×*G. aridum*, D_1D_4, had 12 bivalents and two univalents. The data available (table 1, group 11) indicate that American 13-chromosome species have a slight amount of structural differentiation among their chromosomes.

A number of hybrids between 26-chromosome American, 2(AD), species were studied by WEBBER (1935, 1939). He reported that there are usually 26 bivalents at M I, but sometimes one, two, or three associations of four chromosomes were present, and the chiasma frequency was apparently somewhat reduced. Some of these crosses were studied in the present work, and the results indicate that chromosome irregularities occur more frequently in crosses of *G. hirsutum*×*G. barbadense* and *G. hopi* Lewton×*G. hirsutum* than in pure species.

Octoploids, $2[(AD)_1(AD)_2]$, produced by doubling the chromosome number in F_1 hybrids of *G. hirsutum*, $2(AD)_1$,×*G. barbadense*, $2(AD)_2$, had more than half the chromosomes associated in quadrivalents at M I (Plate

1, fig. E). The average number of quadrivalents in 24 cells was 15.2 or 58 percent of the maximum number. The number of quadrivalents per cell varied from 4 to 23. There was an average of 0.7 trivalents and 0.7 univalents per cell. The average of 15.2 quadrivalents per cell is just slightly lower than the average of 17.5 quadrivalents found in an octoploid of *G. hirsutum*, and the average of 18.1 found in an octoploid of *G. barbadense*. This shows that there is little preferential pairing of the chromosomes in the octoploid from F_1 hybrids of the two species, and it is further evidence that there is little structural differentiation of the chromosomes of *G. hirsutum* and *G. barbadense*.

CHROMOSOME BEHAVIOR IN HYBRIDS AND POLYPLOIDS OF DISTANTLY RELATED SPECIES

Three species of Gossypium—*stocksii*, 2E, of Arabia-India, *sturtii*, 2C, of Australia, and *anomalum*, 2B, of Africa—seem to be distantly related to each other and to all other species. These three species, together with the three groups of species (Asiatic, 2A; American 13-chromosome, 2D; and American 26-chromosome, 2(AD), give six distantly related types of Gossypium (table 2).

American 26- ×Asiatic 13-Chromosome Species

Hybrids of American 26-chromosome, 2(AD), and Asiatic 13-chromosome, 2A, cottons were studied by SKOVSTED (1934), FENG (1935), and WEBBER (1935). These authors usually found 13 to 15 univalents and 12 to 13 bivalents. SKOVSTED's results differed from the others in that he found higher associations to be common (table 1, group 6). Some of SKOVSTED's figures show evidence of bridges. The data collected on this cross in the present work (table 3), though similar to that collected by SKOVSTED, differ in that figures were found with fewer than 13 univalents and the average number of univalents is lower. Most of the present studies were based on crosses of *G. hirsutum*×*G. herbaceum*, although some data were collected on a cross of *G. barbadense*×*G. herbaceum* and one of *G. hirsutum* ×*G. arboreum*. The chromosome behavior in the three crosses differed slightly in number and types of multivalent associations.

Two associations of four chromosomes were common at M I (table 3). At A I the chromosomes sometimes separate without definitely showing bridges, but one to three bridges were common. It is believed that most of the bridges involve chromosomes from the two parent species, since in American 26-chromosome species the duplicated segments within sets, which were shown from the study of haploids to be present, are small in comparison to the segments duplicated in the nearly homologous sets from the different species.

The frequent presence of 13 univalents, 11 bivalents, and two associations of four chromosomes at M I, and of one to three bridges at A I, show that two of the three sets of 13-chromosomes in triploid (AD)A hybrids are similar, but that they differ by at least two translocations and perhaps one or more inversions.

TABLE 3

Chromosome behavior at M I of crosses between American 26- and Asiatic 13-chromosome species.

COMBINATION NUMBER	Number of I*	II	III	IV	V	VI	FRAGMENTS	*G. hirsutum* (NEW BOYKIN) × *G. herbaceum*	*G. hirsutum* (RED LEAF) × *G. herbaceum*	*G. barbadense* (PIMA) × *G. herbaceum*	TOTAL
1	13	13					1		2		2
2	13	13							1	1	2
3	15	12								1	1
4	13	11		1				3	1		4
5	13	11		1			1	1		1	2
6	12	11			1			1			1
7	15	10		1					1		1
8	11	10		2					1		1
9	13	9		2				8	5	4	17
10	13	9		2			1		1		1
11	12	9	1			1				1	1
12	17	9		1					1		1
13	12	8	1	2				1			1
14	11	8	1	1	1				1		1
Total	469	357	3	49	2	1	3	14	14	8	36
Average	13.03	9.92	0.1	1.36	0.05	0.03					

* I—univalent; II—bivalent, etc.

SKOVSTED (1934) emphasized that 26-chromosome species have a set of 13 chromosomes that tend to be larger than the other set of 13. He also noted that it is the larger set from the 26-chromosome species that pairs in the triploid with the set from the 13-chromosome Asiatic species, which corresponds to the larger set in size. This observation of SKOVSTED's was confirmed in the present work.

The chromosome number was doubled in triploids of *G. hirsutum* × *G. herbaceum* to give fertile hexaploids, $2[(AD)_1A_1]$. At M I of these hexaploids some of the chromosomes usually are in complex associations. Figures frequently had one or more univalents, association of three or of four chromosomes, or a combination of the three (fig. 2). Associations of more than four chromosomes were seen.

Since anaphase separation is more important in determining the viability of gametes than M I association, and also tends to reflect the results of M I associations, three A I figures were analyzed to get a more accurate interpretation of chromosome behavior in this hexaploid. In one figure the separation was 40-38, with evidence of one fragment. One anaphase (fig. 3) had 40 chromosome bodies going to each pole. A third A I figure (fig. 4) had several fragments and evidence of bridges. At A I lagging chromosomes, bridges, and fragments are common, but the number of these in a given anaphase is usually only one or two.

The M I and A I behavior indicate that almost every megaspore and microspore is unbalanced. This is expected, for M I and A I chromosome behavior in the triploids shows that two of the three sets of chromosomes are closely related. The chromosome behavior of the hexaploid fits the hypothesis as indicated in the formula $2[(AD)_1A_1]$ of four A sets of chromosomes. On the other hand, the amount of chromosome differentiation evidenced in the triploids has considerable influence on chromosome pairing, for the number of quadrivalents is much less than would be expected if four identical sets were present. Associations of more than four chromosomes indicate that chromosomes not structurally alike will sometimes pair even though completely homologous chromosomes are present; this is known, however, from pairing in natural species.

A hybrid pertinent to the clarification of chromosome relationship of cultivated American 26- and Asiatic 13-chromosome cottons was studied by SKOVSTED (1934). This hybrid had 52 chromosomes, and SKOVSTED had evidence that the hybrid resulted from the fertilization of a diploid Asiatic egg with a reduced gamete from 26-chromosome cotton. At M I this hybrid behaved as if it had three like sets and one differentiated set of chromosomes, for it averaged 15.8 univalents, 5.9 bivalents, 6 trivalents, 1 association of four, 0.4 association of five, and 0.1 association of six chromosomes. This is pertinent evidence that one 13-chromosome set in American 26-chromosome cotton is, in a general way, homologous with the 13-chromosome set in Asiatic cottons.

Plants with essentially the same chromosome composition, $2A_1(AD)_1$, were produced during the present study by pollinating stigmas of an autotetraploid Asiatic plant with pollen of a 26-chromosome species. Studies of these plants have confirmed SKOVSTED'S conclusions. The drawing shown in figure 5 is similar to his drawings.

American 26- ×American 13-Chromosome Species

Hybrids of $2(AD)\times 2D$, 26- ×13- chromosome American species, show a meiotic chromosome behavior similar to that described for $2(AD)\times 2A_2$, American 26- ×Asiatic 13-chromosome species. There are important dif-

ferences, however, in the two types of hybrids. In the cross American 26- ×American 13-chromosome cottons the set of small chromosomes in the 26-chromosome species pairs with the 13-chromosome set from the American 13-chromosome species, whereas in the cross with the Asiatic species the large set pairs with the Asiatic 13-chromosome set. It appeared, however, that all the chromosomes were slightly larger in the triploid of American 26- ×Asiatic 13-chromosome. Crosses between 26- and 13-chromosome American species rarely had associations of more than two chromosomes, although associations of three were found. M I figures with 13 associations of two chromosomes and 13 univalents were common, but figures with less than 13 pairs were also present (Plate 1, fig. F). Several figures with 11 associations of two chromosomes and 17 univalents were found, and one or more fragments were present in some cells. Early A I usually showed two or three bridges, and late A I had about the same number. Figures were found with five bridges and evidence of others; but in some A I figures there was little evidence of bridges. Three $2(AD) \times 2D_2$ crosses used in this study—*G. hirsutum*, *G. barbadense*, and *G. hopi* ×*G. harknessii*—had essentially the same chromosome behavior. A few observations of *G. hirsutum* ×*G. thurberi*, $2D_1$, gave about the same range in the number of univalents.

Several crosses between 26- and 13-chromosome American species studied by SKOVSTED (1937) gave much the same chromosome behavior as that described above, except that he failed to mention the occurrence of chromosome bridges. In some M I figures he found more than 13 associations of two chromosomes. Associations of four chromosomes were rare. In rare figures there were only five to nine associations of two chromosomes. He found a chiasma frequency in bivalents slightly below that of the normal species. From SKOVSTED's data (table 1, group 12) and the present work, it appears that crosses in any combination of 26- and 13-chromosome American species have about the same meiotic chromosome behavior.

Hexaploids, $2[(AD)_1D_2]$, produced by doubling the chromosome number in sterile triploids of *G. hirsutum* ×*G. harknessii*, have a higher fertility and, therefore, apparently a more normal chromosome behavior during meiosis than hexaploids, $2[(AD)_1A_1]$, of American 26- and Asiatic 13-chromosome species. HARLAND (1940) found two hexaploids of the former type to be "fully fertile." In most cells of the $2[(AD)_1D_2]$ type there are univalents, trivalents, associations of three or four chromosomes, or a combination of the three, and in some A I figures one or more bridges are present.

It may be said that the chromosome set in American 13-chromosome species is similar in structure to the small 13-chromosome set in American 26-chromosome species. There are, however, a number of structural differences, perhaps five or more.

American 13- ×Asiatic 13-Chromosome Species

Hybrids between 2A and 2D types, Asiatic 13- and American 13-chromosome species, were reported by SKOVSTED (1937) to average about eight associations of two chromosomes and nine to ten univalents at M I. An occasional association of three or four chromosomes was reported. Chiasma frequency in bivalents averaged less than 1.2 (table 1, group 4). His drawings show chromosome bridges. The only cross available for a study of this type in the present work was *G. arboreum* var. *neglectum*×*G. thurberi*. It was found that chromosome bridges were present in nearly every A I, the number ranging from 0 to 9 and averaging 4.7 (table 4). The difference between the present data and that of SKOVSTED may be explained on the basis that slightly different crosses were studied, or on the fact that the number of bridges varies with different environments, as shown in table 4.

TABLE 4

Chromosome bridges at A I of G. arboreum var. neglectum×G. thurberi.

MATERIAL COLLECTED	NUMBER BRIDGES PER CELL									
	0	1	2	3	4	5	6	7	8	9
Fall, 1937		2	1	6	8	13	12	8	10	2
Summer, 1938	2	6	2	10	3	3		1		
Fall, 1938		2	1	1	6	3	1	2		1
Total	2	10	4	17	17	19	13	11	10	3

Total number cells, 106
Average number bridges, 4.7

In an amphidiploid produced from the hybrid all the chromosomes pair usually as bivalents, but higher associations are frequently present. Some A I figures have no bridges, while others have one to three. If the bridges in the initial hybrid were a physiological effect of hybridization, there should be more attenuated chromosomes at A I in the tetraploid than there were in the initial hybrid.

From the number of univalents, the number of bridges in the F_1, and, the behavior of the chromosomes in the tetraploid produced by doubling the chromosome number in the F_1, it seems safe to conclude that structural differences exist between all the chromosomes of Asiatic 13- and those in American 13-chromosome species.

The tetraploid produced by doubling the chromosome number in a hybrid of Asiatic 13- ×American 13-chromosome species was crossed with American 26-chromosome species. At M I about one-third of the cells have one or two univalents, most of the remaining chromosomes are paired

as bivalents, but most cells have one or more multivalent associations. A cell with more than the average number of irregularities is shown in Plate 1, figure G. In some cells three multivalent associations were found, and sometimes bridges were seen in anaphase figures. MAUER (1938) reported meiosis to be regular in a similar hybrid. The number of multivalents and univalents found in the hybrid $A_2D_1(AD)_1$ supports the statements made earlier in this paper that three or more structural differences exist between American 26- and Asiatic 13-chromosome species of Gossypium and about five structural differences between American 26- and American 13-chromosome species.

American 26- ×Australian 13-Chromosome Species

A cross $2(AD)_1 \times 2C_1$, American 26- and Australian 13-chromosome types, *G. hirsutum*×*G. sturtii*, showed from one to nine, and averaged 5.0, chromosome bridges at A I (Plate 1, fig. H; table 5). Approximately 20 percent of the cells had associations of three chromosomes, but associations of four chromosomes were rare. According to SKOVSTED (table 1, group 10), the average number of bivalents in this cross is 7.6. One of his figures of a similar cross shows five bridges.

Hexaploids, $2[(AD)_1C_1]$, produced by doubling the chromosome number in triploid hybrids, sometimes have 39 pairs of chromosomes, although usually some are in associations of four, and two or more univalents may be present. Ordinarily one to three bridges are present at A I in this hexaploid (Plate 1, fig. I), but bridges are sometimes absent. The behavior of chromosomes in the triploid hybrid of these two species and in the hexaploid produced from the hybrid indicates that all the chromosomes of the two species are structurally differentiated.

TABLE 5

Frequency of bridges in a cross of G. hirsutum×G. sturtii, $2(AD)_1 \times 2C_1$.

Number bridges	1	2	3	4	5	6	7	8	9
Frequency	2	7	11	14	25	15	11	8	1

Total number cells, 94
Average number bridges, 5.0

American 26- ×African 13-Chromosome Species

A cross $2(AD)_2 \times 2B_1$, *G. barbadense*×*G. anomalum*, according to SKOVSTED (1937), had an average of 2.6 associations of two chromosomes and 33.8 univalents (table 1, group 8). The chiasma frequency was 1.05. SKOVSTED's drawing of an A I of this cross has four bridges. WEBBER (1939) from a similar cross, *G. hirsutum*×*G. anomalum*, found the number of associations of two chromosomes to average 10.4.

In the present work the cross *G. barbadense* (Pima) × *G. anomalum* averaged 8.9 bivalents at first metaphase in 34 pollen mother cells. Most of the bivalents were attenuated in anaphase, which indicates that most of the chromosomes that pair have structural differences (Plate 1, fig. J).

A hexaploid $2[(AD)_1B_1]$ from *G. hirsutum* × *G. anomalum* was nearer normal in chromosome behavior than any of the other induced Gossypium polyploids that were studied (Plate 1, fig. K). No irregularities were observed in about half of the M I cells; in others, univalents were the most common irregularity, and more rarely multivalents were present. Sometimes one or more bridges were present at anaphase.

American 26- × Arabia-India 13-Chromosome Species

Skovsted reported the cross $2(AD)_2 \times 2E_1$, *G. barbadense* × *G. stocksii*, to average 37.9 univalents and 0.55 association of two chromosomes (table 1, group 13). The maximum number of associations of two chromosomes found was three. In the present work a hybrid of *G. hirsutum* × *G. stocksii*, $(AD)_1E_1$, in 71 PMC averaged 1.9 bridges at A I. A maximum of five bridges was found. The average number of bridges in this cross probably does not differ significantly from the average number found in haploids of *G. hirsutum*.

Asiatic 13- × Australian 13-Chromosome Species

In a cross $2A_2 \times 2C_1$, *G. arboreum* × *G. sturtii*, Skovsted reported M I figures to average 2.8 univalents, 9.8 associations of two, and the remainder as associations of three or four chromosomes. The number of univalents ranged from 0 to 14 (table 1, group 3). He determined the chiasma frequency as 1.3. His text figure 6 indicates that some of the bivalents form bridges at anaphase. Webber (table 1, group 3) from a similar cross $2A_1 \times 2C_1$, *G. herbaceum* × *G. sturtii*, reported the number of bivalents to average 2.2. These data show that considerable chromosome differentiation exists among the species.

Asiatic 13- × Arabia-India 13-Chromosome Species

Skovsted reported hybrids of $2E \times 2A$, *G. stocksii* × Asiatic species, to have from 4 to 26 univalents with an average of 14.9, and the number of associations of two chromosomes averaged 5.6 with a chiasma frequency of 1.1 (table 1, group 5). In the present work a cross of this type *G. stocksii* × *G. arboreum* at M I of 41 pollen mother cells gave a range of 12 to 26 univalents and averaged 3.8 associations of two chromosomes. Although some typical bridges were found and fragments were seen in a small number of cells, most of the associations of two chromosomes were not as attenuated as the bridges in other hybrids. Doubling the chromosome num-

ber in this hybrid gave a polyploid of almost normal fertility. Apparently there is little homology among the A and E sets of chromosomes.

Asiatic 13- ×African 13-Chromosome Species

In crosses between Asiatic 13- ×African 13-chromosome species, 2A×2B, SKOVSTED determined the average number of univalents as 2.2, of bivalents as 11.4, and found a few associations of three and four chromosomes. The range of univalents was 0 to 9 (table 1, group 2). WEBBER reported similar data. A hybrid *G. arboreum* var. *neglectum*×*G. anomalum* studied in this work gave essentially the same averages. There is much homology between A and B sets of chromosomes, but four or more structural differences exist.

Australian 13- ×American 13-Chromosome Species

WEBBER studied four 2D×2C crosses, American 13-×Australian 13-chromosome species, and SKOVSTED studied two of the crosses (table 1, group 9). WEBBER reported an average of about one association of two chromosomes, while SKOVSTED reported an average of about 8.5 associations of two chromosomes in his crosses. WEBBER and SKOVSTED show similar differences in their results from a study of *G. barbadense*×*G. sturtii* and for other hybrids. SKOVSTED's results on a similar cross, *G. hirsutum*×*G. sturtii*, agree with the author's data given earlier in this paper for that cross. It is believed the differences between WEBBER's data and that of other workers is the result of WEBBER's interpretation of chromosome bridges involving pairs of chromosomes as fragmenting univalents. The most logical way to interpret his figures, in the light of the present work, is to assume that the extra chromosome bodies in his drawings are fragments resulting from crossing over between relatively inverted segments of paired chromosomes. SKOVSTED's figures (table 1, group 9) show several bridges. From the data available there can be little doubt that every chromosome in *G. sturtii* differs in structure from every chromosome in American 13-chromosome species.

African 13- ×American 13-Chromosome Species

Crosses of 2D×2B, between three American 13-chromosome species and the African species, *G. anomalum*, were reported by SKOVSTED (table 1, group 7) to have an average number of univalents varying from 14 to 21, depending upon the American 13-chromosome species involved; the average number of associations of two chromosomes varied from 2.4 to 5.7. Associations of three chromosomes were rare. SKOVSTED's figures give evidence that bridges are formed at A I. WEBBER reported the number of associations of two chromosomes to average 0.2. AMIN (1940) reported a

hybrid of *G. davidsonii*×*G. anomalum* to be fertile after its chromosome number was doubled with colchicine. In a hybrid of *G. davidsonii*×*G. anomalum* the author found an average of 3.8 bivalents and a maximum of eight bridges. These data indicate that B and D sets are well differentiated.

Other Combinations

Crosses between some combinations have not been studied, for attempts to produce them either failed or the seedlings died before they reached the flowering stage (SKOVSTED 1935b). These combinations are 2E×2C, Arabia-India (*G. stocksii*)×Australian (*G. sturtii*); 2B×2E, African (*G. anomalum*)×Arabia-India; and 2C×2B, Australian×African.

RELATIONSHIPS BETWEEN THE TYPES OF GOSSYPIUM

In the section devoted to the cytological behavior of hybrids and polyploids, the available detailed cytological evidence was cited on the relationships of the species of Gossypium, but the details obscured the relationships within the genus as a whole.

Failure to secure crosses has prevented a cytological study of four combinations of crosses between the six types. For each absent combination it is possible, as pointed out by SKOVSTED (1937), to cite dubious evidence from triangular relationships of species. He stated, "Consequently it appears reasonable that two species B and C may have originated from a species A and developed in different directions, so that B and C ultimately lose more of their cytological homology with each other than with the parental species A." This is possible if the parent species changes at a slower rate than the derived species. The results may be invalid if two species arose from a common ancestor, and one of the species later branched to give a third. Since it is known that chromosome pairing is under genic control, it is dangerous to judge relationships from triangular associations alone.

In one triangle made up of Asiatic, Australian, and American 13-chromosome species the number of bivalents differs by a minimum of 3.3 in crosses between any combination of two of the three species, but in another triangle involving a different American 13-chromosome species the number of bivalents differs by as much as six (fig. 6). In the triangle, Asiatic, African, and American 13-chromosome species, the frequency of bivalents varies in the three crosses (fig. 6). It is obvious that triangular associations give little definite information about the relationship of species.

The relationships among the six types are summarized in figure 6. It is apparent that the types have different degrees of similarity. In some instances different combinations of crosses between members of two types

give different results. Crosses between members of a type show few or no chromosome structural differences. In the American 13-chromosome type the data are inadequate. The lack of evidence that the chromosomes of *G. davidsonii* and *G. klotzschianum* have several structural differences from the chromosomes of *G. thurberi*, *G. harknessii*, etc., is the reason the American 13-chromosome species are here considered as one type in place of two as suggested by HARLAND (1939).

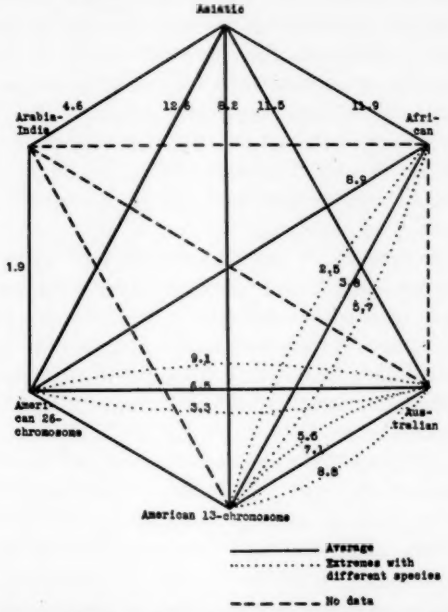

FIGURE 6.—Number of bivalents (number of chromosomes associated divided by two) in crosses between different types of Gossypium. Data from SKOVSTED (1933, 1935, 1937) except for American 26- × African, and American 26- × Arabia-India, which are from the present work.

As shown by an average of 2.4 univalents, 11.3 associations of two chromosomes, and a few associations of more than two chromosomes at M I, the Asiatic type, 2A, *G. arboreum* and *G. herbaceum*, and the African type, 2B, *G. anomalum*, have the closest relationship that exists among the six types. From SKOVSTED's data, the Asiatic type and the Australian type, 2C, *G. sturtii*, are slightly more distantly related, since there is an average of 2.8 univalents, 9.8 associations of two chromosomes, and a few multivalent associations. American 13-chromosome species, 2D, are next in closeness of relationship with Asiatic species, there being 9.6 univalents, 7.8 associations of two chromosomes, and rarely an association of three or

four chromosomes. An average of 4.7 bridges at A I shows that the paired chromosomes in the hybrid usually are structurally differentiated. Based on an average of 16.6 univalents and 4.6 associations of two chromosomes in hybrids between the Arabia-India type, 2E, *G. stocksii* × Asiatic species, it appears that *G. stocksii* is more distantly related to the Asiatic species than are the other types; on the other hand, if any consideration is given to the triangular relationships shown in figure 6, the Asiatic type is more closely related to *G. stocksii* than are any other types.

Although relationships of the African type, 2B, to Asiatic species, 2A, are the closest of any of the types, the differences are particularly apparent in crosses involving the two species with a third. For example, *G. anomalum*, 2B, averaged 18.5 univalents and 3.8 associations of two chromosomes in crosses with American 13-chromosome species, 2D, while the comparable number of bivalents in 2A × 2D crosses was 8.2 (fig. 6). Further, African × American 26-chromosome crosses averaged 3.6 bivalents less than American 26-chromosome × Asiatic.

The Australian type, 2C, *G. sturtii*, averaged 8.2 and 5.3 associations of two chromosomes at M I in crosses with different American 13-chromosome species, 2D. *G. sturtii* is doubtlessly more closely related to Asiatic species than to American 13-chromosome species. The relationship between Australian and Arabia-India species, 2E, is apparently distant, and it may approach the differences existing between *G. stocksii* and Asiatic species plus the differences between *G. sturtii* and Asiatic species (fig. 6).

There are indications that the degree of relationship of American 13-chromosome species, 2D, with Australian, 2C, Asiatic, 2A, and African species, 2B, decreases in the order given. There is no direct evidence available on the relationship of American 13-chromosome species with the Arabia-India species, 2E, *G. stocksii;* however, it is possible that these two are the most distantly related of the six types. The evidence for this is an average of only 1.9 associations of two chromosomes in a cross of *G. stocksii* with an American 26-chromosome species, 2(AD).

SKOVSTED (1937) divided 13-chromosome Gossypium species into an American and an Old World group. WEBBER (1939) separated the Old World group into Australian and Asiatic. The data shown in figure 6 and the relationships pointed out above show it to be erroneous to include all Old World species in one or even in two types. There is evidence of less homology between chromosomes of certain of SKOVSTED'S (1937) and WEBBER'S (1939) Old World types than there is between some Old World types and New World species. For example, the average number of associations of two chromosomes in crosses between the Arabia-India, 2E, species, *G. stocksii*, and Asiatic, 2A, species is 4.6, while the number in a cross between American 13-chromosome species, 2D, and Asiatic species is 8.2.

The relationship and origin of American 26-chromosome species remains to be considered. As shown in a previous section, two 13-chromosome sets in hybrids between American 2(AD) 26- and Asiatic, 2A, 13-chromosome species tend to associate as bivalents or higher multiples. The larger 13-chromosome set in American 26-chromosome species pairs with the set of similar size from Asiatic species. There is, however, a minimum of three structural differences in chromosomes between the two sets that pair. In hybrids of 26-chromosome species with an autotetraploid Asiatic type, the chromosomes in three 13-chromosome sets tend to associate as trivalents.

In crosses between American 26- and American 13-chromosome species, two 13-chromosome sets tend to associate as bivalents; but in this case it is the smaller 13-chromosome set that associates with a set of similar size from an American 13-chromosome species. There is a minimum of four structural differences between the sets of chromosomes that pair.

These facts, summarizing chromosome behavior in hybrids between American 26-chromosome and Asiatic and in hybrids between American 26-chromosome and American 13-chromosome types, except for the structural differences between the chromosomes that pair, were noted by SKOVSTED (1934). He advanced a hypothesis that American 26-chromosome species are allotetraploids, which arose from a hybrid between American 13- and Asiatic 13-chromosome species. SKOVSTED (1937) reported an average of 7.8 bivalents in a hybrid between Asiatic 13- and American 13-chromosome species. GATES (1938) cast some doubt on SKOVSTED's hypothesis and suggested that 26-chromosome cottons could be either autotetraploids or allotetraploids involving two American 13-chromosome species. WEBBER (1939) discussed the published evidence of the origin of American 26-chromosome cottons.

Obviously, the most critical evidence in this matter is the pairing of the two sets of chromosomes in American 26-chromosome species in crosses with tetraploids synthesized from an American 13- and an Asiatic 13-chromosome species (BEASLEY 1940b). Since usually only one or two, and frequently no, univalents are present at M I in hybrids of this type and the hybrids are partly fertile, there is no doubt that one set of chromosomes in the American tetraploid 26-chromosome species is similar in structure to the set in American 13-chromosome species and that the other set is similar to the set in cultivated Asiatic species. As SKOVSTED (1934) doubtlessly appreciated, and as pointed out by MAUER (1938), there is no evidence that any existing 13-chromosome species is exactly like those involved in American tetraploid species.

MAUER, from geographic distribution and genetic differences among the 26-chromosome species of Gossypium, suggested independent origins for

the species and that different 13-chromosome species were the parents of the allotetraploids. Geographical distribution does not support the idea of separate origins, for extremes of the tetraploid type are native in the New World. The behavior of the meiotic chromosomes of the tetraploid synthesized from species similar to the ones that gave rise to the American 26-chromosome species strongly supports the idea that all the natural tetraploid Gossypium species came from one original tetraploid plant. The evidence is that the synthesized tetraploid has a rather high frequency of chromosome irregularities. If separate lines were established from the initial progeny of the tetraploid, they would probably differ in chromosome structure in a manner similar to the different lines of Triticale examined by MÜNTZING (1939). The fact that meiotic chromosome behavior is regular in hybrids between the natural tetraploids is excellent evidence that the natural 26-chromosome species not only had a common origin but also that a single line from the original tetraploid was evolved with regular chromosome behavior and it later branched to give the existing natural tetraploid species.

The relationships of Gossypium species give an indication of the center of origin or distribution of the genus. Asia is suggested, for Asiatic species are apparently intermediate in the relationships among other types. To judge from the available evidence, the Arabia-India type is more closely related to Asiatic species than to other types. It is, however, more distantly related to Asiatic species than Asiatic species are to other types. The Australian, African, and American 13-chromosome types are more closely related to Asiatic species than to each other, with the possible exception that American 13-chromosome cottons are about equally distantly related to Asiatic and Australian types.

CHANGES IN CHROMOSOME STRUCTURE AND SPECIATION

Ample evidence has been given to show that there are many differences in chromosome structure among the types of Gossypium. For example, pollen mother cells were found with nine bridges (involving 18 chromosomes) in the cross *G. arboreum* var. *neglectum*×*G. thurberi*. It is probable that the other eight chromosomes failed to pair because the structural differences between them were too numerous. The maximum number of bridges found in a cross between the Australian and the American 26-chromosome type was also nine.

It is the author's opinion that in any diploid hybrid that shows half or less than half of the chromosomes in associations of two, the bivalents will usually give bridges at anaphase, for the reduction in the number of bivalents is believed to result usually from structural differences between

chromosomes (DOBZHANSKY 1937). It follows that in hybrids with a sufficient number of structural differences to cause the failure of half the chromosomes to pair, it is probable that one or more structural differences exists between paired chromosomes. Gene combinations, asynaptic genes, are known to prevent or greatly reduce chromosome pairing, but that type of chromosome behavior is not considered to hold here, because the pairing of the chromosomes in polyploids from the hybrids is almost normal. Doubling the chromosome number in asynaptic plants is not followed by normal chromosome pairing (unpublished data).

The extent of structural differences in the chromosomes of different species of Gossypium may be divided into four classes. The first class includes types lacking any apparent differences in chromosome structure; for example, *G. hirsutum*, $2(AD)_1 \times$ *G. barbadense*, $2(AD)_2$. The second class includes types that approach the normal number of associations, but a few bridges, univalents, or multivalents, or combinations of the three, are present at A I. An example of this type is *G. arboreum* var. *neglectum* 2A × *G. anomalum*, 2B. In the next type there is a reduced and variable number of associations of two chromosomes, and nearly every chromosome association gives a bridge at A I. The cross *G. hirsutum*, $2(AD) \times$ *G. sturtii*, 2C (table 1, group 10, and table 5), is an example. The fourth class consists of species crosses in which the structural differences among chromosomes are so numerous that almost no chromosome association is present, as illustrated by *G. hirsutum*, $2(AD) \times$ *G. stocksii*, 2E.

The present problem is to evaluate the importance of chromosome structural changes in the speciation of Gossypium and to attempt to account for the existence of the changes.

It may be that chromosome structural changes are of incidental importance compared to gene mutation and that similar species differences would exist today if the chromosomes had undergone no rearrangement, provided the species had remained isolated by other factors and polyploid species were excluded. Some evidence that speciation in Gossypium is independent of chromosome structural changes is seen from the species grouped within the general types. These species show little chromosome differentiation, yet they differ so greatly in leaf size, shape, flowers, physiology, etc., that there is no doubt of their being distinct species. The American 13-chromosome type contains species that were originally classified into three genera: *G. thurberi* Tod. = *Thurberi thespesiodes* A. Gray; *G. armourianum* Kearney = *G. armourianum* Kearney; *G. aridum* (Rose and Standley) Skovsted = *Erioxylum aridum* Rose and Standley. The American 26-chromosome type has species ranging from small herbs to perennial shrubs or trees, and they show other outstanding differences, yet in crosses between

species, they are all inter-fertile and exhibit almost normal chromosome behavior. In these cases isolation was probably geographic, and gene changes were of major importance in the differentiation of species.

There appear to be differences in chromosome structure between each of the six types of Gossypium, but, since the American 26- and the American 13-chromosome types include distinct species with few or no structural differences among their chromosomes, it appears that most of the structural differences that exist among the six types became established after the species were separated. The apparent fact that initial speciation in Gossypium is by gene changes does not mean that changes in chromosome structure were unimportant in the speciation of the genus. The appearance of an allotetraploid type is probably the greatest single step in speciation of Gossypium, and this could hardly have occurred without chromosome structural differentiation among species.

Since chromosome structural changes in Gossypium are seemingly of little importance in initial speciation, it may be assumed that they are unimportant in the evolution of a single species. It is probable that gene changes arise and become established at a much higher rate than chromosome changes, which gives them greater initial importance. It is likely, however, that chromosome changes occur that are also important steps in the evolution of a species, for, as pointed out by DARLINGTON (1937, 1940), they could give types of variation (usually duplication of genes and perhaps position effect) that could hardly arise by other means.

The work of DOBZHANSKY and STURTEVANT (DOBZHANSKY 1937) shows that changes in chromosome structure are frequent in Drosophila and that they are important in the evolution of species. STEBBINS (1938) found changes in chromosome structure to be important in the isolation of species, but if species were isolated by other means, as great or greater species differences could exist without changes in chromosome structure.

At present it is a question how the chromosome structural changes in Gossypium became established. An initial structural change should be a disadvantage, since it would be heterozygous, and many of the gametes would be inviable. Since there is usually more self- than cross-pollination in Gossypium, individuals homozygous for the chromosome change could be produced, but the type would be in a minority; and unless changes in chromosome structure possessed survival value, the type ordinarily would be lost, or it would be carried along in the population like a gene with no survival value. If the population were small and large fluctuations were frequent, at times by chance structurally changed types would replace original types. The survival of types by chance, rather than by survival of the fittest, is discussed by DOBZHANSKY (1937).

Structural changes have occurred in the chromosomes of the six types of Gossypium mentioned above, and they have survived and supplanted earlier chromosome types. The rate at which this chromosome differentiation can become established is slow. This in no way means that changes in chromosome structure are rare, for the frequency with which they are found in nature shows that they are relatively common (SANSOME and PHILP 1939). The rapidity with which structural changes are incorporated into species is an entirely different matter. If changes in chromosome structure possess survival value, the selection pressure for their survival, after they become homozygous in a species that is mostly self-pollinated, would be the same as that for a gene of equal survival value.

These structural chromosome changes have a significance in regard to the supposition that 13-chromosome cottons are primitive polyploids. This idea was advanced by DAVIE (1935) and by WEBBER (1935) and was supported by SKOVSTED (1937). The idea could be correct, but basing the argument on the secondary pairing found in some 13-chromosome cottons is objectionable. Obviously, since so many structural changes have occurred in Gossypium, it is almost certain that there are duplications within a basic chromosome set. The autosyndesis reported by SKOVSTED (1935) is cytological evidence that duplications are present. These duplications could be the cause of much secondary pairing. DARLINGTON (1937) gives a general discussion of such possibilities.

Considering the evidence available, it appears that the first step in the speciation of Gossypium is the geographical or physiological isolation from the general population. Gene mutation and different gene combinations arise rapidly, and some of them become established at a comparatively rapid rate. Detectable structural changes in the chromosomes arise occasionally, and some of them become established, but at a much slower rate.

SUMMARY

The meiotic chromosome behavior is regular in 13-chromosome species of Gossypium, but there are a few irregularities in 26-chromosome species. In meiosis of autopolyploids about two-thirds of the chromosomes form quadrivalents. In a haploid of a 26-chromosome species a maximum of five pairs of chromosomes was found.

In hybrids between species the amount of chromosome pairing varies from complete to almost none. In hybrids with a reduced amount of pairing there was evidence that structural differences existed among some of the chromosomes, and in some species hybrids apparently all the chromosomes were structurally dissimilar. Most of the chromosomes formed bivalents in polyploids that were produced from hybrids with a reduced

amount of chromosome pairing; usually, however, cells in first metaphase and anaphase had one or more anomalies.

The tetraploid species of Gossypium have one set of chromosomes similar to the set in Asiatic 13-chromosome species and the other set similar to the set in American 13-chromosome species.

The species of Gossypium are separated into six general types chiefly on the basis of chromosome pairing, structure (arrangement of the genes), and chromosome number. The degree of relationship of the types is discussed.

Structural changes in the chromosomes probably had little importance in the initial speciation of Gossypium.

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THE ORIGIN OF ^{h}DECIPIENS FROM THE COMPLEXES OF OENOTHERA LAMARCKIANA AND ITS BEARING UPON THE PHYLOGENETIC SIGNIFICANCE OF SIMILARITIES IN SEGMENTAL ARRANGEMENT[1]

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IN PREVIOUS papers on Oenothera (CLELAND 1931, 1935, 1937), it has been suggested that genomes or complexes which resemble each other in segmental arrangement owe this similarity in most cases to a relatively recent common ancestry. On this assumption, similarity in segmental arrangement is, in this genus, a probable indication of close phylogenetic relationship. Thus, for example, two complexes whose segmental arrangements are such that one could be derived theoretically from the other by two interchanges probably, in many or most cases, do differ from each other by this minimal amount—that is, one has come from the other by a process involving but two interchanges, or else they have come from a common ancestor, each as a result of a single but different interchange.

It has been emphasized all along, however, that this hypothesis must be applied with proper reserve. It would be a mistake to assume that degree of resemblance in segmental arrangement is always an exact measure of phylogenetic relationship. In most cases it probably has such significance, but in some cases it may not. There doubtless are cases where more complicated histories of interchange have brought about a resemblance in segmental arrangement through convergence, which is therefore of minor phylogenetic significance. The mathematical probability of such an occurrence, however, is slight, and such cases are no doubt exceptional. Nevertheless, the possibility of their occurrence must not be lost sight of.

In the present paper, one of these presumably exceptional cases will be discussed. This case is presented, not as a refution of the hypothesis that similarity in segmental arrangement is in general of phylogenetic significance, but as an example of the sort of complication which may occur and which should be kept in mind. ^{h}*Decipiens* apparently differs from ^{h}*hookeri* by a single interchange, yet it is known to have been derived from the complexes of *lamarckiana* by a process involving not fewer than two interchanges and therefore cannot be so closely related to ^{h}*hookeri* from the phylogenetic point of view as it would appear at first sight to be. Since this is an instructive case, the possibilities of its origin will be canvassed in some detail.

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ʰDecipiens is one of the complexes of *erythrina*, a so-called "half-mutant" derived from *Oe. lamarckiana*. *Erythrina* contains the segmentally unaltered, lethal-bearing *velans* of *lamarckiana* and the mutant, alethal complex, *ʰdecipiens*. The latter segregates out from *erythrina* in each generation, giving the "full" mutant *decipiens* (*ʰdecipiens*·*ʰdecipiens*). *ʰDecipiens* is believed to contain a mixture of genes from *velans* and *gaudens*, since its phenotypic effect is intermediate between that produced by the two complexes of *lamarckiana*.

Erythrina has arisen independently on numerous occasions in the cultures of both DE VRIES and SHULL. Our particular strain was obtained from PROF. B. M. DAVIS and was derived from SHULL'S so-called "1910-selfed" line of *erythrina*. This line has had a complicated ancestry involving two strains of *lamarckiana* and a line of "*biennis*" (private correspondence). In spite of this fact, it is apparently identical in the segmental arrangements of its complexes with the *erythrina* (derived from DE VRIES) used by CATCHESIDE and others, as is shown when the chromosome configurations which it gives with various complexes are compared with those given by CATCHESIDE'S *erythrina* (CATCHESIDE 1939a). In all probability, our *erythrina* arose in the same manner as the other *erythrinas*, and the reasoning based on our *erythrina* will apply to the others also.

Our problem, then, is to learn, if possible, how *ʰdecipiens* has arisen from *lamarckiana* and how it may be related to the complexes of this race. This becomes a possibility for the first time because of the fact that the segmental arrangements of *velans* (one of the complexes of *lamarckiana*) and of *ʰdecipiens* have been determined and the possibilities for the segmental arrangement of *gaudens* (the other complex of *lamarckiana*) have been reduced to two (CLELAND and HAMMOND, unpublished). The segmental arrangements of these complexes are as follows:

velans = 1·2	3·4	5·8	7·6	9·10	11·12	13·14
ʰdecipiens = 1·2	3·4	5·6	7·14	9·10	11·12	13·8
gaudens = 1·2	3·11	5·6	7·12	9·4	8·14	13·10
1·2	3·11	5·6	7·13	9·14	10·4	12·8

Since the relationships among the three complexes are identical on the basis of either alternative possibility for *gaudens*, it will make no differenee for the purposes of this paper which formula for *gaudens* is chosen. We shall therefore adopt the first alternative, remembering that the other ultimately may prove to be the correct one.

With these formulae as a basis, how can *ʰdecipiens* have arisen from the complexes of *lamarckiana?*

A superficial comparison of *ʰdecipiens* with *velans* and *gaudens* suggests a much closer affinity to *velans* than to *gaudens*. The *ʰdecipiens* arrange-

ment could not be derived from the *gaudens* arrangement by fewer than four interchanges, whereas it could be derived from *velans* by no more than two interchanges. Has *ʰdecipiens* actually come from *velans* by a series of two interchanges, or has it arisen by some other route?

First, it should be recalled that *erythrina* (and hence, presumably, *ʰdecipiens*) was thought by DE VRIES to have arisen from *lamarckiana* in one step. Is this possible? Reasoning *a priori*, it is obvious that this could have happened only if the 5·6 of *ʰdecipiens* is the 5·6 of *gaudens* and if the 1·2 3·4 9·10 11·12 chromosomes have come from *velans;* if *ʰdecipiens* acquired its 5·6 chromosome from *gaudens* and these other chromosomes from *velans*, then perhaps a single interchange might bring into existence the two other *ʰdecipiens* chromosomes which differ from *velans*—namely, 7·14 and 8·13. An examination of the formulae for *velans* and *gaudens*, however, shows that no single interchange, whether between two *velans*, two *gaudens*, or a *velans* and *gaudens* chromosome, could bring into existence simultaneously both of these chromosomes. We are forced to conclude, therefore, that *ʰdecipiens* did not arise from *lamarckiana* as a result of a single interchange. At least two interchanges must have occurred in succession. In the following discussion we shall assume that *ʰdecipiens* arose from the *lamarckiana* genomes by a series of two interchanges. While it is possible that it arose by a more roundabout process, the chances are probably against this and, furthermore, it is not necessary to our present purpose to point out every way by which *ʰdecipiens* might conceivably have arisen.

The reasoning which is to follow has been worked out both on the assumption that the process of interchange takes place at the four-strand stage and on the assumption that it may occur at a time prior to this stage. The same results are obtained in either case, so that it is immaterial for the present argument whether the mechanism of interchange is reciprocal translocation or crossing over. We shall therefore assume that the interchanges involved in the creation of *ʰdecipiens* have occurred at the four-strand stage.

We may first ask which of the *ʰdecipiens* chromosomes have come directly from *gaudens* or *velans* and which are the result of interchange. There is one chromosome in *ʰdecipiens* which is common to both *gaudens* and *velans*—namely, 1·2. This constitutes the pair in *lamarckiana*. Obviously, the pair has not been involved in the interchanges leading to the *ʰdecipiens* arrangement. Another chromosome in *ʰdecipiens* is 5·6. This could have been derived directly from *gaudens* or, as we shall see, it could theoretically have been produced by interchange from *velans* chromosomes. It will be shown below, however, that any complex with the *ʰdecipiens* end arrangement which has derived its 5·6 from *velans* will contain only *velans* ends and hence, because of the lethals, will no doubt be unable to function

in the same plant with the unaltered *velans* to produce *erythrina*. *Decipiens* therefore must have received its 5·6 from *gaudens*.

Decipiens has three chromosomes whose end arrangements are identical with *velans* chromosomes (3·4, 9·10, 11·12). If *decipiens* has arisen as a result of only two interchanges, then it must have derived these three chromosomes directly from *velans*, for if one of these is created by an interchange between *gaudens* chromosomes or between a *gaudens* and a *velans* chromosome, it then becomes impossible to form the remaining *decipiens* chromosomes which differ from both *velans* and *gaudens* by the single remaining interchange. Therefore, we must assume that *decipiens* has received all three of these chromosomes direct from *velans*.

Summarizing to this point, we may say that if *decipiens* has been derived by a succession of two interchanges, it must have received the following chromosomes direct from *lamarckiana* without interchanges being involved: 1·2 (from the pair), 5·6 (from *gaudens*), 3·4, 9·10, 11·12 (from *velans*). There remain but two *decipiens* chromosomes to be accounted for, and these, which differ both from *gaudens* and *velans* in segmental arrangement, must have been formed by interchange. These two chromosomes are 7·14 and 13·8. They cannot both be formed simultaneously by a single interchange between any of the chromosomes in *lamarckiana;* they must have been created by a succession of at least two interchanges. What sort of interchange could have produced them?

It is clear that these interchanges have involved only those chromosomes which contain one or more of the following ends: 7, 14, 13, and 8 (those ends found in 7·14 and 13·8). The *velans* chromosomes which may have been concerned are 5·8, 7·6, 13·14; the *gaudens* chromosomes which could have been involved are **7·12**, **14·8**, **13·10**.[2] The initial exchange could have involved two *velans* chromosomes, two *gaudens* chromosomes, or a *velans* and a *gaudens* chromosome. We shall consider each of these possible types of initial interchange in turn.

First, we shall discuss cases in which the initial exchange is assumed to have involved two *velans* chromosomes. This interchange might have been between 5·8 and 13·14, or 7·6 and 13·14, or 7·6 and 5·8.

I(A). EXCHANGE BETWEEN 5·8 AND 13·14, TO GIVE 5·14 AND 13·8[3]

The sporocyte in which interchange occurred should in this case show at metaphase the condition seen in figure 1,[4] unless the chromosomes on one side of the point of interchange should become reversed in their position with respect to the poles of the spindle, a condition which would not

[2] *Gaudens* ends will be in boldface type wherever they appear in the text.

[3] In this and in all following cases we shall assume regularity in the zigzag arrangement in the first metaphase.

[4] Dashed lines represent *gaudens* chromosomes or ends, solid lines represent *velans*.

lead to the "*ʰdecipiens*" end arrangement. Assuming that non-disjunction does not occur in the second division (this will be assumed in all cases below), one of the four resulting spores and the gamete derived from it could have the complex:[5] 1·2 3·4 5·14 7·6 9·10 11·12 13·8.

The gamete carrying this complex would have only *velans* ends and hence would not be able in all probability to unite with *velans;* it would have to unite with *gaudens*, with which it would give ⊙12, 1 pair.

In F_1 or a subsequent generation, one of four interchanges (*vide infra*) might occur to produce the *ʰdecipiens* arrangement, and the gamete thus formed would have to unite with an unaltered *velans* gamete if it were to produce *erythrina*. This could be accomplished in this case only by outcrossing (the union would not have to occur immediately, since *ʰdecipiens* could be carried along indefinitely in association with *gaudens*).

1. Suppose 5·14 and 7·6 exchanged to give 5·6 and 7·14. The sporocyte in which this interchange occurred might show at metaphase the condition seen in figure 2. One of the four resulting spores, and the gamete derived from it, would have the complex 1·2 3·4 **5·6** 9·10 11·12 7·14 13·8 (=*ʰdecipiens*). If disjunction in the first anaphase occurred strictly in accordance with the above diagram, all ends in this "*ʰdecipiens*" complex would be *velans* ends except for **5·6**. If the *velans* lethal is in ends 5 or 6 and the *gaudens* lethal is not, this might, by union with an unaltered *velans*, give *erythrina*. On the other hand, the chromosomes on one side of the interchange could be oriented in the reverse direction in the spindle. This could yield, as before, one spore with the *ʰdecipiens* arrangement, but this time the 5·6 would belong to *velans*. Since all of its ends would be *velans* ends, it is unlikely that this complex could combine with an unaltered *velans* to form *erythrina*, on account of the lethals.

2. 5·14 **7·12** might exchange to give 5·**12** **7**·14. The sporocyte in which this interchange occurred might show at metaphase the condition seen in figure 3 (reversal of orientation on one side of the point of interchange would not lead to the *ʰdecipiens* end arrangement). If disjunction occurred on the two sides of the point of interchange as indicated in the figure, one

[5] In Oenothera, there is a strong tendency in the first telophase for associated chromatids of a dyad to remain attached at the centric region, but to separate widely along the remainder of the chromosome, each dyad taking on the appearance of a Maltese cross. It is quite probable, therefore, that chromatids 13·8 and 5·8 (in this case) would remain together in interkinesis and would separate from chromatids 5·14 and 13·14. In the second anaphase, either chromatid of one dyad could pass into the same spore with either chromatid of the other dyad, viable spores being produced if they receive 5·14 and 13·8 or 5·8 and 13·14; inviable spores being formed if they receive 5·14 and 5·8 or 13·8 and 13·14. Many of the interchanges discussed further on in this paper would result in a similar situation in the spores.

FIG. 1

FIG. 2

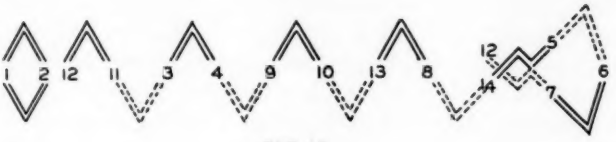


FIG. 3

FIG. 4

FIG. 5

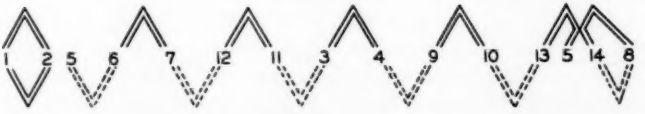


FIG. 6

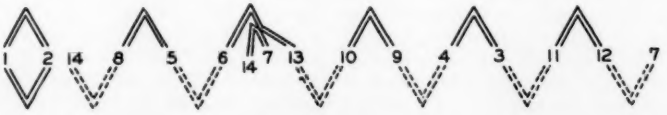


FIG. 7

spore of the quartet would have the *ʰdecipiens* arrangement as follows: 1·2 3·4 **5·6 7**·14 9·10 11·12 13·8. If the *velans* lethal is in ends 5, 6, or 7, and the *gaudens* lethal is not, this might, by union with *velans*, form *erythrina*. In this case *ʰdecipiens* would have ends 5, 6, or 7 of *gaudens*.

3. **8·14** 7·6 might exchange to give 7·**14** 6·**8**. The sporocyte in which this interchange occurred might show at metaphase the condition seen in figure 4 (reversal of orientation would not lead to the *ʰdecipiens* arrangement). If disjunction occurred on the two sides of the point of interchange as indicated, one spore of the quartet would carry a *ʰdecipiens* arrangement as follows: 1·2 3·4 **5·6** 7·14 9·10 11·12 13·8. If the *velans* lethal is in ends 5, 6, or 14 and the *gaudens* lethal is not, this could unite with *velans* to form a presumptive *erythrina*, whose *ʰdecipiens* would have ends 5, 6, and 14 of *gaudens*.

4. **8·14 7·12** might exchange to give **7·14 8·12**. If disjunction occurred on the two sides of the point of interchange as indicated in figure 5 (reversal of orientation on one side of the point of interchange would not lead to the *ʰdecipiens* arrangement), one spore of the quartet would have the *ʰdecipiens* arrangement, with ends 5, 6, 7, and 14 of *gaudens:* 1·2 3·4 **5·6 7·14** 9·10 11·12 13·8. If the *velans* lethal is in ends 5, 6, 7, or 14, and the *gaudens* lethal is not, this "*ʰdecipiens*" might, by union with the *velans* from another plant, produce *erythrina*.

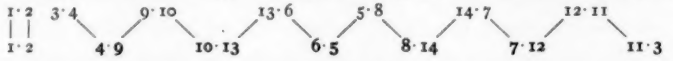
We find, therefore, that there are four different ways in which a succession of two interchanges could produce a *ʰdecipiens* end arrangement from *lamarckiana*, assuming that the initial interchange was between 5·8 and 13·14 of *velans* to form 5·14 13·8. We find that the "*ʰdecipiens*" complexes formed in each of the four ways would differ in the number of *gaudens* ends present. Ends 5 and 6 of *gaudens* would be present in all, and 7 or 14 (or both of these) might also be present.

I(B). EXCHANGE BETWEEN 5·8 AND 13·14, GIVING 5·13 AND 8·14

The chromosomes on the two sides of the point of interchange could be oriented as shown in figure 6, or those on one side might be inverted in position. In either case, the interchange complex would have the formula 1·2 3·4 5·13 7·6 9·10 11·12 8·14 (or **8·14**). If 8·14 is present, the ends of this complex would all be *velans* ends, and the resultant gamete would no doubt have to unite with *gaudens* in order to function, giving ⊙10, 2 pairs. If **8·14** is present, the complex could possibly function with *velans*. In either case, however, an interchange complex with this formula would not, by a single exchange of segments within itself, or by an exchange with *gaudens* or *velans*, be able to produce a complex with the *ʰdecipiens* formula. It is therefore very unlikely that the exchange between 5·8 13·14 to give 5·13 8·14 was involved in the production of *ʰdecipiens*.

II(A). EXCHANGE BETWEEN 7·6 AND 13·14 TO FORM 7·14 AND 13·6

With the chromosomes arranged as in figure 7 (reversal of orientation on one side of the point of interchange would produce spores all of which would have certain segments in duplicate and would lack others), one of the types of viable spore and gamete that could result would have the formula 1·2 3·4 5·8 7·14 9·10 11·12 13·6. Since all ends in this cell would be *velans* ends, this gamete would no doubt have to unite with a *gaudens* gamete in order to function, thus producing a plant with ⊙12, 1 pair.



From this beginning, a complex with the *h decipiens* arrangement could be obtained by any one of four interchanges:

1. 5·8 13·6 might exchange to give 5·6 13·8. If the chromosomes were oriented as shown in figure 8, one of the resultant gametes would have the *h decipiens* formula, but this would have entirely *velans* ends and hence would probably not be able to function with *velans*. If the chromosomes on one side of the point of interchange were reversed in their orientation toward the poles, a gamete with the *h decipiens* arrangement would still be produced, but this would contain ends 5 and 6 from *gaudens*. If the *velans* lethal is in ends 5 or 6, and the *gaudens* lethal is not, this could unite with a *velans* gamete from another plant, and the result might be *erythrina*.

2. 5·8 **13·10** might exchange to give **13**·8 5·**10**. Assuming the orientation of chromosomes shown in figure 9, one of the spores produced from this sporocyte would have the *h decipiens* arrangement of ends, with ends 5, 6, and 13 of *gaudens*. 1·2 3·4 **5·6** 7·14 9·10 11·12 **13**·8. If the *velans* lethals are in ends 5, 6, or 13, and the *gaudens* lethals are not, the gamete produced by this spore might function with *velans* to give *erythrina*. Should the chromosomes on one side of the point of interchange be reversed in their orientation to the poles, the spores bearing the interchange chromatids would be inviable due to the presence of deficiencies and duplications, and the viable spores would not have the *h decipiens* arrangement.

3. **8·14** 13·6 might exchange to give 13·**8** 6·**14**. Assuming the orientation shown in figure 10, one of the types of viable spores produced from this sporocyte would have the *h decipiens* arrangement of ends, with ends 5, 6, and 8 of *gaudens*. 1·2 3·4 **5·6** 7·14 9·10 11·12 13·**8**. If the *velans* lethals are in ends 5, 6, or 8 and the *gaudens* lethals are not, this complex might function with *velans* to give *erythrina*. Were the chromosomes on one side of the point of interchange reversed in their position with reference to the poles, a gamete with the *h decipiens* arrangement could not be produced.

4. **8·14 13·10** might exchange to give **13·8 10·14**. With the chromo-

FIG. 8

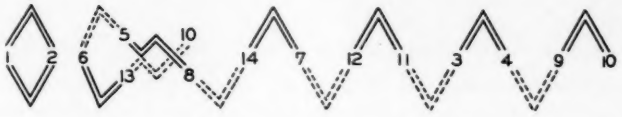


FIG. 9

FIG. 10

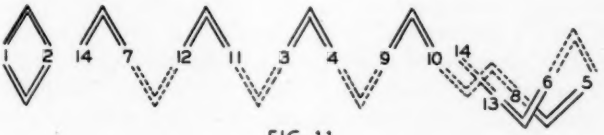


FIG. 11

FIG. 12

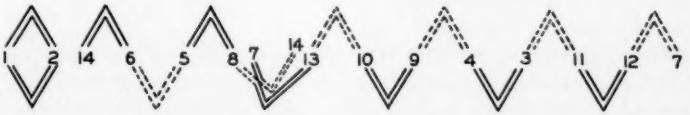


FIG. 13

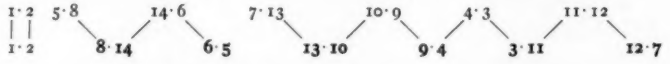
somes oriented as shown in figure 11, one spore out of four would have the *decipiens* end arrangement. 1·2 3·4 **5·6** 7·14 9·10 11·12 **13·8**. The gamete derived therefrom would be able to function with *velans* to produce *erythrina*, provided the lethal of *velans* is in ends 5, 6, 8, or 13, and the *gaudens* lethal is absent from these ends. A reversal of orientation of the chromosomes on one side of the point of interchange would make the immediate production of the *decipiens* end arrangement impossible.

No other exchanges would produce a *decipiens* end arrangement following the initial exchange of 7·6 13·14 to form 7·14 13·6.

II(B). EXCHANGE BETWEEN 7·6 AND 13·14 TO GIVE 7·13 AND 6·14

Assuming the orientation shown in figure 12, the gamete receiving the interchange chromatids would have the following segmental arrangement: 1·2 3·4 5·8 7·13 9·10 11·12 6·14.

Since this gamete would have only *velans* chromosomes, it would have to unite with a *gaudens* gamete; the resultant plant would have ⊙4, ⊙8, 1 pair:



This plant would probably not breed true with respect to chromosome configuration. In either a plant with ⊙4, ⊙8 or a plant derived from a gamete carrying the *gaudens* half of the ⊙4 and the *velans* half of the ⊙8 combined with either *velans* or *gaudens*, the further exchange of 7·13 **8·14** to give 7·**14** 13·**8** (fig. 13) would be possible, producing a *decipiens* arrangement with 5, 6, 8, and 14 of *gaudens*. 1·2 3·4 **5·6** 7·**14** 9·10 11·12 13·**8**.

This complex, combined with *velans*, might produce an *erythrina*, provided the *velans* lethal is in ends 5, 6, 8, and 14 and the *gaudens* lethal is not. (Reverse orientation on one side of the point of interchange during the second exchange would not result in the *decipiens* arrangement.)

Had the chromosomes been oriented in reverse fashion on one side of the point of exchange during the first interchange, the gamete receiving the interchange chromatids would have had the formula: 1·2 3·4 **5·6** 7·13 9·10 11·12 **8·14** (fig. 14).

This complex could combine with either *velans* or *gaudens*, assuming the lethals to be placed as indicated below. A further interchange between 7·13 and **8·14** to give 7·**14** and **8**·13 would yield the *decipiens* arrangement which again would have 5, 6, 8, and 14 of *gaudens*. This could presumably produce *erythrina* if the *velans* lethal is in ends 5, 6, 8, or 14 and the *gaudens* lethal is not.

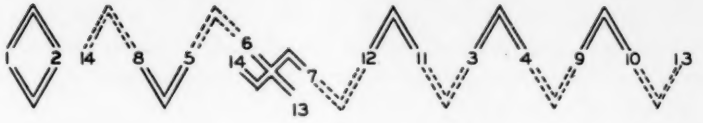


FIG. 14

FIG. 15

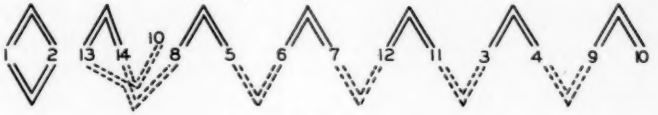


FIG. 16

FIG. 17

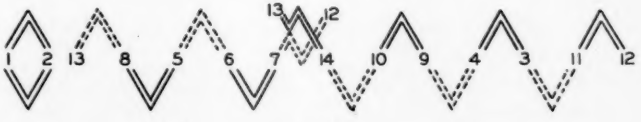
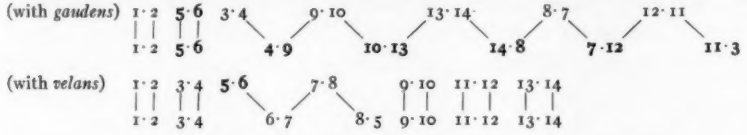


FIG. 18

FIG. 19

III(A). EXCHANGE BETWEEN 7·6 AND 5·8 TO FORM 5·6 and 7·8

With the chromosomes oriented as in figure 15, a spore would be produced with the formula 1·2 3·4 **5·6** 7·8 9·10 11·12 13·14. A gamete produced from this spore could unite with *gaudens* if the *gaudens* lethal is not in 5 or 6, or could unite with *velans* if the lethal of the latter is in 5 or 6.



In either case, a further interchange between 7·8 and 13·14, giving 7·14 and 13·8, could produce a complex with the *[h]decipiens* arrangement, capable of functioning with *velans* to form a plant with the *erythrina* formulae, provided the *velans* lethals are in 5·6 and the *gaudens* lethals are not.

If the chromosomes were oriented in the reverse manner on one side of the point of exchange at the time of the initial interchange, the gamete with the interchange chromatids would have only *velans* segments and would therefore have to unite with *gaudens*. It would have the formula 1·2 3·4 5·6 7·8 9·10 11·12 13·14. A further exchange between 7·8 and 13·14 would give the *[h]decipiens* arrangement, and there would be a 50 percent chance that the gamete receiving the interchange chromatids in this case would get **5·6** instead of 5·6, in which case it might unite with *velans* to form *erythrina*, if the *velans* lethal is in 5·6 and the *gaudens* lethal is not.

III(B). EXCHANGE BETWEEN 7·6 AND 5·8 TO FORM 5·7 AND 6·8

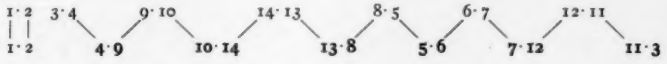
A complex formed by this interchange could not be transformed into one with the *[h]decipiens* end arrangement by a single additional interchange, and hence it is unlikely that this particular exchange has been involved in the production of *[h]decipiens*.

This completes the possible ways by which *[h]decipiens* might have arisen through a succession of two interchanges, assuming that the initial interchange was between two *velans* chromosomes. Next we may consider the possibilities assuming that the initial interchange was between two *gaudens* chromosomes. This exchange might have been between **8·14** and **13·10**, or **7·12** and **13·10**, or **8·14** and **7·12**.

IV(A). EXCHANGE BETWEEN 8·14 AND 13·10 TO GIVE 13·8 AND 10·14

With the chromosomes oriented as shown in figure 16 (reverse orientation would produce only spores containing duplications and deficiencies), one type of viable spore which could result from this cell would contain: 1·2 **3·11 5·6 7·12 9·4 10·14 13·8**.

This complex, having only *gaudens* ends, would have to unite with *velans*.



From this combination, a *hdecipiens* arrangement could be produced in one of four ways:

1. **7·12 10·14** might exchange to give **7·14 10·12**.

If the chromosomes disjoined as shown in figure 17, one of the four spores would have the *hdecipiens* end arrangement, with ends 5, 6, 7, 8, 13, and 14 of *gaudens*. 1·2 3·4 **5·6 7·14** 9·10 11·12 **13·8**. This complex might (by either open or self-pollination) unite with *velans* to form *erythrina*, assuming that the *velans* lethals were in 5, 6, 7, 8, 13, or 14 and the *gaudens* lethals were not. Had the chromosomes been oriented in the reverse direction on one side of the point of interchange, *hdecipiens* would not have been formed as a result of this interchange. The fact should be emphasized that in this case a formula with the *hdecipiens* arrangement has been formed without *velans* chromosomes having been involved in either exchange. Although *hdecipiens* seems at first sight to have been derived from *velans* as a result of two interchanges, it could also have resulted from two interchanges between *gaudens* chromosomes.

2. 13·14 **7·12** might exchange to give 13·**12** 7·14.

Assuming that the chromosomes are disjoined as shown in figure 18, one of the two types of viable spore which could result would have the *hdecipiens* end arrangement, with ends 5, 6, 7. 8, and 13 of *gaudens*. 1·2 3·4 **5·6 7**·14 9·10 11·12 **13·8**. This complex might (by open or self pollination) unite with *velans* to form *erythrina*, assuming the *velans* lethals to be in ends 5, 6, 7, 8, or 13 and the *gaudens* lethals to be absent from these. Had the chromosomes on one side of the point of interchange been oriented in the reverse manner, *hdecipiens* would not have been formed by this interchange.

3. 13·14 7·6 might exchange to form 7·14 13·6.

If the chromosomes were oriented as shown in figure 19, one of the viable spores which could be formed would contain the *hdecipiens* arrangement of ends, having ends 5, 6, 8, and 13 of *gaudens*. 1·2 3·4 **5·6** 7·14 9·10 11·12 **13·8**. If the *velans* lethal is in these ends and the *gaudens* lethal is not, the gamete containing this complex would be able to unite with *velans* (by open or self pollination), and the result might be *erythrina*. A reversal in orientation of the chromosomes on one side of the point of attachment, however, would make it impossible to form *hdecipiens* from this exchange.

4. **10·14** 7·6 might exchange to give 7·**14** 6·**10**.

Assuming the orientation shown in figure 20 one of the types of viable

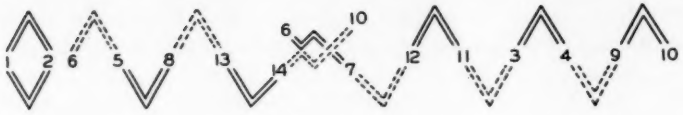


FIG. 20

FIG. 21

FIG. 22

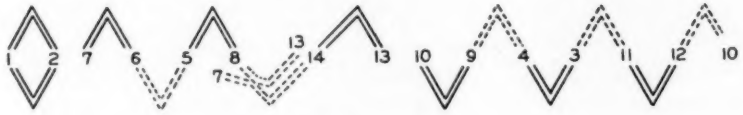


FIG. 23

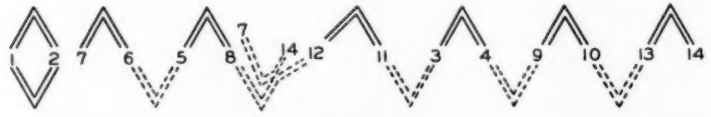


FIG. 24

FIG. 25

spore which could be derived from the sporocyte experiencing this interchange would have the *ʰdecipiens* end arrangement, with 5, 6, 8, 13, and 14 of *gaudens*. 1·2 3·4 **5·6** 7·14 9·10 11·12 **13·8**. This would give rise to a gamete capable of uniting with *velans* (by open or self pollination) to produce a presumptive *erythrina*, provided that the *velans* lethals are in ends 5, 6, 8, 13, or 14 and the *gaudens* lethals are not. Reversal of orientation of the chromosomes on one side of the point of attachment would make it impossible for a *ʰdecipiens* to be formed directly as a result of this interchange.

IV(B). EXCHANGE OF 8·14 AND 13·10 TO FORM 8·10 AND 13·14

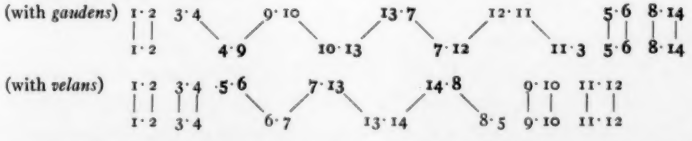
A complex arising in this manner would not be able to produce a complex with the *ʰdecipiens* arrangement by a single additional interchange, involving either its own chromosomes or one or more chromosomes of *velans*. Hence it is improbable that the exchange of **8·14 13·10** to give **8·10 13·14** has been involved in the production of *ʰdecipiens*.

V(A), EXCHANGE OF 7·12 AND 13·10 TO FORM 7·10 AND 13·12

With the chromosomes oriented as in figure 21, no spore could be produced which would give the *ʰdecipiens* arrangement after one additional interchange. On the other hand, reversal of orientation on one side of the interchange would result in spores all of which would have some segments in duplicate and would lack others and which in all probability would be functionless. The exchange of **7·12 13·10** to give **7·10 13·12** is therefore unlikely to have been involved in the evolution of the *ʰdecipiens* end arrangement

V(B). EXCHANGE OF 7·12 AND 13·10 TO FORM 7·13 AND 10·12

With the chromosomes oriented as in figure 22, a spore could be formed bearing the following arrangement: 1·2 3·4 **5·6** 7·13 9·10 11·12 **8·14**. This could unite with either *gaudens* or *velans*, assuming the lethals to be properly placed.

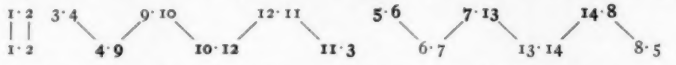


No matter with which of these it united, a further interchange between **7·13** and **8·14** in a subsequent generation (the only interchange which would give the desired results) would produce the *ʰdecipiens* end arrangement in which ends 5, 6, 7, 8, 13, and 14 would have come from *gaudens*. 1·2 3·4 **5·6 7·14** 9·10 11·12 **13·8**. A gamete with this arrangement,

upon union with a *velans* gamete might give *erythrina*, assuming the *velans* lethals to be in ends 5, 6, 7, 8, 13, or 14 and the *gaudens* lethals to be absent from these ends.

The reverse orientation of the chromosomes on one side of the point of interchange, in the case of the initial interchange, could give as one of the spores: 1·2 **3·11 5·6 7·13 9·4 10·12 8·14**.

This, upon union with *velans*, would give a plant with ⊙6, ⊙6, 1 pair.



An interchange between **7·13** and **8·14** would yield *h decipiens*, providing the proper half of the circle of six not involved in the interchange came to reside in the spore carrying the exchange chromatids (fig. 23).

The resulting *h decipiens* would be identical in respect to the ends carried with that obtained by the alternative orientation at the time of the initial interchange, as shown above.

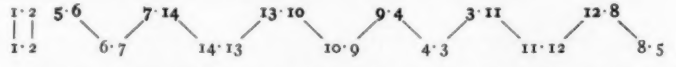
In this and the preceding case, the interchanges producing the *h decipiens* arrangement have involved only *gaudens* chromosomes.

VI(A). EXCHANGE OF 8·14 AND 7·12 TO GIVE 8·12 AND 7·14

The sporocyte in which this interchange occurred would show at metaphase the condition seen in figure 24 (no other orientation would be possible without resultant non-disjunction).

One of the viable spores which could result from this sporocyte would have the end arrangement: 1·2 **3·11 5·6 7·14 9·4 13·10 8·12**.

The gamete arising from this could unite with *velans*.



In a subsequent generation, one of four interchanges might occur, any of which would be capable of producing the *h decipiens* arrangement.

1. **5·8 13·10** might exchange to give **13·8 5·10**.

With the orientation on the two sides of the point of interchange shown in figure 25, one of the types of viable spores which might arise would have the *h decipiens* arrangement, with 5, 6, 7, 13 and 14 of *gaudens*. 1·2 3·4 **5·6 7·14** 9·10 11·12 **13·8**. If the *velans* lethals are in these ends and the *gaudens* lethals are not, the complex found in this spore might combine with *velans* to produce *erythrina*. Reverse orientation of the chromosomes on one side of the point of interchange would not yield the *h decipiens* arrangement.

2. **8·12 13·10** might exchange to give **13·8 10·12**

With the chromosomes oriented as in figure 26, one of the types of viable spore resulting would have the *h decipiens* arrangement, with 5, 6, 7, 8, 13,

FIG. 26

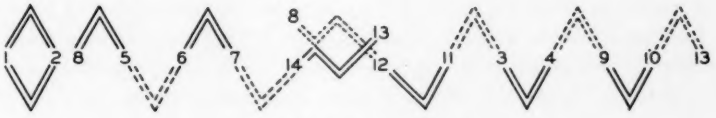


FIG. 27

FIG. 28

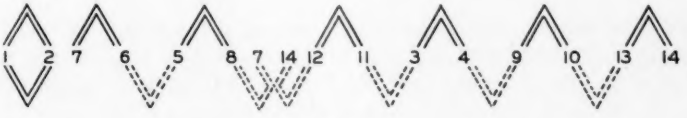


FIG. 29

FIG. 30

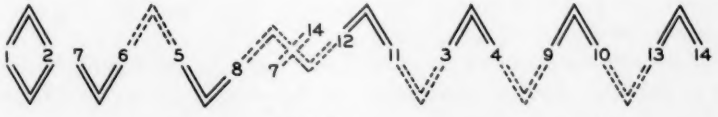


FIG. 31

and 14 of *gaudens*. 1·2 3·4 **5·6 7·14** 9·10 11·12 **13·8**. Assuming that the *velans* lethal lies in one of these ends, but not the *gaudens* lethal, this complex might unite with *velans* to form an *erythrina*. Reverse orientation of the chromosomes on one side of the point of interchange would not yield the *decipiens* arrangement.

3. **8·12** 13·14 might exchange to give 13·**8** **12**·14.

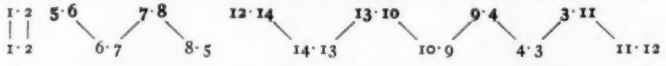
With the orientation shown in figure 27, one spore could be produced with the *decipiens* arrangement, having 5, 6, 7, 8, and 14 of *gaudens*. 1·2 3·4 **5·6 7·14** 9·10 11·12 13·**8**. This complex would combine with *velans* to form an *erythrina*, assuming that the *velans* lethal is in these ends and the *gaudens* lethal is absent from these ends. Reverse orientation of the chromosomes on one side of the point of interchange would not yield the *decipiens* arrangement.

4. 5·8 13·14 might exchange to give 5·14 13·8.

With the chromosomes oriented as in figure 28, one of the spores produced would have the *decipiens* end arrangement, with 5, 6, 7, and 14 of *gaudens*. 1·2 3·4 **5·6 7·14** 9·10 11·12 13·8. This complex might unite with *velans* to produce *erythrina* provided the *velans* lethal is in 5, 6, 7, or 14, and the *gaudens* lethal is not in these ends.

VI(B). **8·14 7·12** MIGHT EXCHANGE TO GIVE **7·8 12·14**

Assuming the orientation shown in figure 29, the gamete receiving the interchange chromatids would have the formula: 1·2 **3·11 5·6 7·8 9·4 12·14 13·10**. This complex would have to unite with *velans*, since it has only *gaudens* ends. The resultant plant would have ⊙4, ⊙8, 1 pair.



A second exchange between **7·8** and 13·14 in this plant (fig. 30) or any subsequent individual which retained the *gaudens* half of the ⊙4 and the *velans* half of ⊙8 would make possible the production of a gamete carrying the *decipiens* arrangement of segments, in which are included 5, 6, 7, and 8 of *gaudens*. 1·2 3·4 **5·6 7·14** 9·10 11·12 13·**8**. This complex could unite with *velans* to produce an "*erythrina*," if the *velans* lethals are in 5, 6, 7, 8 and the *gaudens* lethals are not.

If the chromosomes had the reverse orientation on one side of the point of exchange in the first interchange, the following complex could be produced (fig. 31): 1·2 3·4 **5·6 7·8** 9·10 11·12 13·14.

This could unite with either *velans* or *gaudens* if the lethals are placed as indicated below. In either case, a second exchange between **7·8** and 13·14 would make possible a gamete carrying the *decipiens* arrangement, which would include 5, 6, 7, 8 of *gaudens*. This complex might unite with *velans*

FIG. 32

FIG. 33

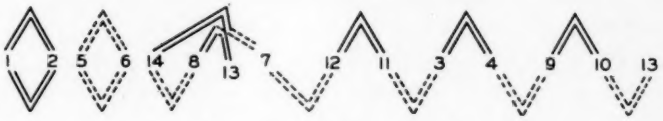


FIG. 34

FIG. 35

FIG. 36

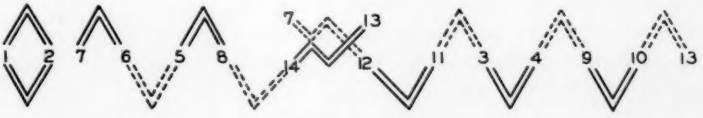


FIG. 37

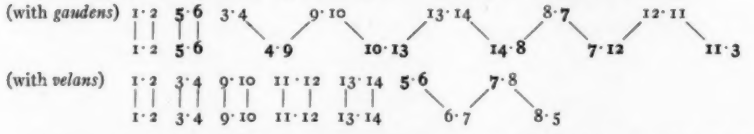
to produce *erythrina*, if the *velans* lethals are in 5, 6, 7, 8 and the *gaudens* lethals are not.

This completes the possible ways by which *decipiens* (and *erythrina*) could have been evolved, through the medium of two interchanges, assuming that the initial interchange was one involving two *gaudens* chromosomes. We may lastly consider the possibilities, assuming that the initial exchange was between a *velans* and a *gaudens* chromosome.

VII(A). EXCHANGE OF 5·8 AND 7·12 TO GIVE 5·12 AND 7·8

Assuming the orientation found in figure 32 (reversal of orientation would not yield viable interchange complements), one of the resulting spores could have the following end arrangement: 1·2 3·4 **5·6** **7·8** 9·10 11·12 13·14.

This complex could unite with either *velans* or *gaudens*, assuming that the *velans* lethal is in ends 5, 6, or 7, and the *gaudens* lethal is not.



A second exchange (the only one possible) between **7·8** and 13·14 to give **7·14** and 13·8 might in either case (fig. 33, 34) result in a complex with the *decipiens* arrangement, carrying 5, 6, and 7 of *gaudens*. 1·2 3·4 **5·6** **7·14** 9·10 11·12 13·8. This, united to *velans*, might produce an *erythrina*, assuming that the *velans* lethal is in 5, 6, or 7 and the *gaudens* is not. Reversal of orientation on one side of the point of interchange would not in these cases produce the *decipiens* arrangement.

VII(B). EXCHANGE OF 5·8 AND 7·12 TO GIVE 5·7 AND 8·12

In this case it would not be possible to produce the *decipiens* arrangement by a single additional interchange.

VIII(A). EXCHANGE OF 5·8 AND 13·10 TO GIVE 5·13 AND 8·10

Spores receiving the interchange chromatids would in this case possess complexes from which the *decipiens* arrangement could not arise, no matter whether *velans* or *gaudens* were associated with these complexes. Therefore, this exchange is not likely to have been involved in the production of *decipiens* (fig. 35).

VIII(B). EXCHANGE OF 5·8 AND 13·10 TO GIVE 5·10 AND 13·8

Spores receiving the interchange chromatids would in this case possess duplications and deficiencies and in all probability would therefore prove inviable. Hence, this exchange cannot have been involved in the production of *decipiens* (fig. 36).

FIG. 38

FIG. 39

FIG. 40

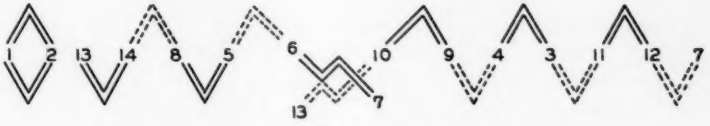


FIG. 41

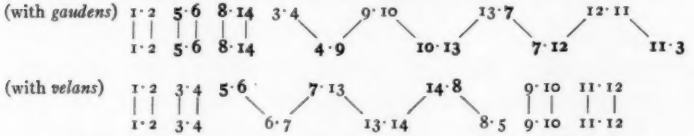
FIG. 42

FIG. 43

IX(A). EXCHANGE BETWEEN 13·14 AND 7·12 TO FORM 7·13 AND 12·14

Assuming that the chromosomes are oriented as in figure 37, one of the spores which can be produced would have the formula 1·2 3·4 **5·6** 7·13 9·10 11·12 **8·14**.

The gamete arising from this spore might unite with either *gaudens* or *velans* if the assumption regarding lethals mentioned below is correct.



Assuming the orientation shown in figures 38 and 39, a second interchange (the only one capable of producing *decipiens*) between 7·13 and 8·14 to give 7·14 and 13·8 would produce the *decipiens* end arrangement, containing 5, 6, 7, 8, and 14 of *gaudens*. 1·2 3·4 **5·6 7·14** 9·10 11·12 13·**8**. A gamete with this arrangement might unite with *velans* to produce *erythrina*, provided the *velans* lethal is in ends 5, 6, 7, 8, or 14 and the *gaudens* lethal is not.

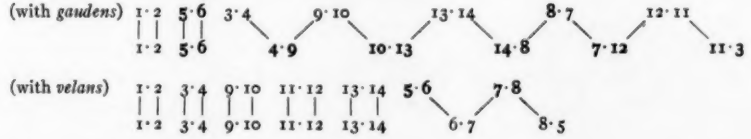
IX(B). EXCHANGE BETWEEN 13·14 AND 7·12 TO FORM 13·12 AND 7·14

No matter which way the chromosomes were oriented in this case, the spores containing the interchange chromatids would show duplications and deficiencies and hence would probably be inviable. This exchange, therefore, would not lead to the production of *decipiens*.

X(A). EXCHANGE BETWEEN 7·6 AND 8·14 TO FORM 7·8 AND 6·14

Assuming the orientation shown in figure 40 (reversal would yield inviable interchange gametes), one of the viable gametes would contain the following segmental arrangement: 1·2 3·4 **5·6 7·8** 9·10 11·12 13·14.

This could unite with either *velans* or *gaudens*, if the *velans* lethals are in 5, 6, or 8 and the *gaudens* lethals are not.



A further interchange in either case between 7·8 and 13·14 would yield a complex with the *decipiens* arrangement, having 5, 6, and 8 of *gaudens*. This might function with *velans* to produce *erythrina* provided the *velans* lethals are in 5, 6, or 8 and the *gaudens* lethals are not.

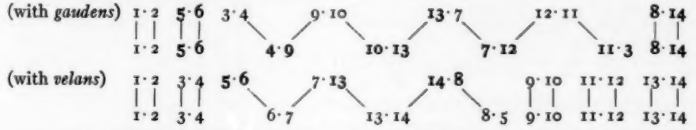
X(B). EXCHANGE BETWEEN 7·6 AND 8·14 TO FORM 7·14 AND 6·8

No matter which way the chromosomes were oriented in this case, the spores containing the interchange chromatids would show duplications and deficiencies and hence would probably be inviable. This exchange, therefore, could not lead to the production of *ʰdecipiens*.

XI(A). EXCHANGE BETWEEN 7·6 AND 13·10 TO FORM 7·13 AND 6·10

With the chromosomes oriented as shown in figure 41 (the reverse orientation would not produce viable spores containing interchange chromatids), a complex could be formed with the following formula: 1·2 3·4 **5·6** 7·13 9·10 11·12 **8·14**.

If the lethals are situated as assumed below, this complex could unite with either *gaudens* or *velans*.



A second interchange between 7·13 and **8·14** to give **7·14** and **13·8** (fig. 42, 43). would produce the *ʰdecipiens* arrangement, with 5, 6, 8, 13, and 14 of *gaudens*. 1·2 3·4 **5·6** 7·14 9·10 11·12 **13·8**. If the *velans* lethal lies in ends 5, 6, 8, 13, and 14 and the *gaudens* lethal does not, this interchange complex might unite with *velans* to form *erythrina*.

XI(B). EXCHANGE BETWEEN 7·6 AND 13·10 TO FORM 7·10 AND 13·6

No matter which way the chromosomes were oriented in this case, the spores containing the interchange chromatids would show duplications and deficiencies and hence would probably be inviable. This exchange, therefore, could not lead to the production of *ʰdecipiens*.

Of the four remaining possible initial interchanges between a *velans* and a *gaudens* chromosome containing ends 7, 8, 13, or 14, none could pave the way for the production of the *ʰdecipiens* arrangement after a second interchange, with the possible exception of the exchange between 13·14 and 8·14 to give **8·13** and **14·14**. This interchange would result in non-disjunction and the formation of a ring univalent (14·14) and it is very doubtful (although theoretically possible) whether the normal *ʰdecipiens* arrangement would be produced, even if the proper second interchange were to take place. The possibility of its formation is so remote that it will be ignored.

DISCUSSION

The origin of ʰdecipiens

We have now described the various ways by which *ʰdecipiens* could theoretically have arisen from *lamarckiana* by a process involving two

TABLE 1

Summary of interchanges capable of producing the ʰdecipiens end arrangement from velans and gaudens. In each case, the velans and gaudens ends present in the resultant ʰdecipiens complex are shown, as well as the position of velans and gaudens lethals. Segments 1 and 2 are omitted from consideration, since these are in the pair in lamarckiana, which has not been involved in the production of ʰdecipiens.

SEQUENCE OF INTERCHANGES PRODUCING ʰ*decipiens* END ARRANGEMENT	THE RESULTANT ʰ*decipiens* END ARRANGEMENT HAS	
	gaudens ENDS (these numbers also indicate the *velans* ends which could carry the *velans* lethals)*	*velans* ENDS (these numbers also indicate the *gaudens* ends which could carry the gaudens lethals*·†
I(a) 5·8 13·14→5·14 13·8		
5·14 7·6→5·6 7·14	5, 6	3, 4, 7, 8, 9, 10, 11, 12, 13, 14
5·14 **7·12**→5·12 7·14	5, 6, 7	3, 4, 8, 9, 10, 11, 12, 13, 14
7·6 **8·14**→7·14 6·8	5, 6, 14	3, 4, 7, 8, 9, 10, 11, 12, 13
8·14 7·12→7·14 **8·12**	5, 6, 7, 14	3, 4, 8, 9, 10, 11, 12, 13
II(a) 7·6 13·14→7·14 13·6		
5·8 13·6 → 5·6 13·8	5, 6	3, 4, 7, 8, 9, 10, 11, 12, 13, 14
5·8 **13·10**→13·8 5·10	5, 6, 13	3, 4, 7, 8, 9, 10, 11, 12, 14
8·14 13·6 →13·8 6·14	5, 6, 8	3, 4, 7, 9, 10, 11, 12, 13, 14
8·14 13·10→13·8 **10·14**	5, 6, 8, 13	3, 4, 7, 9, 10, 11, 12, 14
II(b) 7·6 13·14→7·13 6·14		
7·13 8·14→7·14 13·8	5, 6, 8, 14	3, 4, 7, 9, 10, 11, 12, 13
III(a) 7·6 5·8→5·6 7·8		
7·8 13·14→7·14 13·8	5, 6	3, 4, 7, 8, 9, 10, 11, 12, 13, 14
IV(a) **8·14 13·10**→13·8 **10·14**		
7·12 10·14→ 7·14 **10·12**	5, 6, 7, 8, 13, 14	3, 4, 9, 10, 11, 12
13·14 **7·12**→13·12 7·14	5, 6, 7, 8, 13	3, 4, 9, 10, 11, 12, 14
13·14 7·6 → 7·14 13·6	5, 6, 8, 13	3, 4, 7, 9, 10, 11, 12, 14
10·14 7·6 → 7·14 6·10	5, 6, 8, 13, 14	3, 4, 7, 9, 10, 11, 12
V(b) **7·12 13·10**→7·13 **10·12**		
7·13 8·14→7·14 13·8	5, 6, 7, 8, 13, 14	3, 4, 9, 10, 11, 12
VI(a) **8·14 7·12**→**8·12 7·14**		
5·8 **13·10**→13·8 5·10	5, 6, 7, 13, 14	3, 4, 8, 9, 10, 11, 12
8·12 13·10→13·8 **10·12**	5, 6, 7, 8, 13, 14	3, 4, 9, 10, 11, 12
8·12 13·14→13·8 **12·14**	5, 6, 7, 8, 14	3, 4, 9, 10, 11, 12, 13
5·8 13·14→ 5·14 13·8	5, 6, 7, 14	3, 4, 8, 9, 10, 11, 12, 13
VI(b) **8·14 7·12**→**7·8 12·14**		
7·8 13·14→7·14 13·8	5, 6, 7, 8	3, 4, 9, 10, 11, 12, 13, 14
VII(a) 5·8 **7·12**→5·12 **7·8**		
7·8 13·14→7·14 13·8	5, 6, 7	3, 4, 8, 9, 10, 11, 12, 13, 14
IX(a) 13·14 **7·12**→7·13 12·14		
7·13 8·14→7·14 13·8	5, 6, 7, 8, 14	3, 4, 9, 10, 11, 12, 13
X(a) 7·6 **8·14**→7·8 6·14		
7·8 13·14→7·14 13·8	5, 6, 8	3, 4, 7, 9, 10, 11, 12, 13, 14
XI(a) 7·6 **13·10**→7·13 6·10		
7·13 **8·14**→7·14 13·8	5, 6, 8, 13, 14	3, 4, 7, 9, 10, 11, 12

* See footnote 6 for argument in support of assumption that *velans* and *gaudens* lethals are not in homologous segments.

† Since ʰ*decipiens* is alethal, the *velans* ends which were lost in the production of ʰ*decipiens*, being exchanged for *gaudens* ends, were obviously the ones carrying the *velans* lethals, and the *gaudens* ends received by ʰ*decipiens* were just as obviously ends which could not carry the *gaudens* lethals.

interchanges (reciprocal translocation or crossing over). The 24 different possibilities leading to 14 genetically different complexes with the ʰ*decipiens* arrangement are found in table 1.

Although this table presents the total number of theoretically possible

ways of forming *ʰdecipiens* by a succession of two interchanges, it should not be concluded that the actual number of possibilities for the origin of *ʰdecipiens* is necessarily as high as 24. It must be remembered that each of the above described routes by which *ʰdecipiens* might have arisen depends upon the *velans* and *gaudens* lethals being placed in a particular way. If the lethals are placed in one way, certain possibilities exist for the origin of *ʰdecipiens;* if they are placed in a different way, other possibilities exist. For instance, it may be seen from the table that an altered complex with the *ʰdecipiens* arrangement could arise in only ten ways if the *velans* lethal lies in segment 13;[6] it could have arisen in 12 ways if this lethal is in segment 7; in 14 ways if it is in segment 8, and in all 24 ways if the lethal is in segments 5 or 6. Hence, unless the *velans* lethal is in ends 5 or 6, an alethal complex with the *ʰdecipiens* arrangement could not actually have arisen in as many as 24 ways. No matter where the lethals are placed, however, there are a number of different ways (ten or more) by which it might have arisen, one of which has resulted in *ʰdecipiens*.

But if this be the case, why should it not be possible for more than one kind of alethal complex with the *ʰdecipiens* arrangement to arise? The answer is that other complexes of this type have without doubt actually arisen. Thus, *deserens* (of *rubrinervis*), which has arisen frequently in DE VRIES' cultures (DE VRIES 1919) gives ⊙6 with *velans* (CLELAND 1925). From the standpoint of its phenotypic effect, this complex differs from *ʰdecipiens* in only one respect—namely, in the production of brittle stems. From the standpoint of segmental arrangment, it is apparently identical with *ʰdecipiens*, since a derivative from it, apparently by gene mutation (*rubricalyx* α), has been shown (CATCHESIDE 1939b) to have the *ʰdecipiens* arrangement. *Deserens* and *ʰdecipiens* probably differ merely in the number of *gaudens* ends they carry; they probably represent the result of two of the several different ways by which an alethal complex with the *ʰdecipiens* end arrangement may arise. It is probable also that *rubrisepala* (derived from the Swedish *lamarckiana*—HÅKANSSON 1926) is a mutant having another such complex. This complex also gives ⊙6 with *velans*, and the half mutant it produces with *velans* resembles *erythrina* very closely. In all probability, therefore, more than one series of interchange has actually brought

[6] It is assumed that the *gaudens* lethal is not in the same segment as the *velans* lethal, for if it were, it would be virtually impossible to obtain an alethal complex from *lamarckiana* with an arrangement of ends different from those found in *velans* and *gaudens*. Such a complex could be obtained only in the event that a reciprocal translocation, or crossover between homologous segments in otherwise non-homologous chromosomes, were to follow a crossover which transferred the lethal of one complex to the other complex without the corresponding transfer of the lethal of the other complex taking place. The chances of such a succession of events would seem to be much too slight to account for the relatively frequent appearance of *ʰdecipiens* and other alethal complexes identical with or similar to *ʰdecipiens* in segmental arrangement.

into being the *hdecipiens* arrangement of ends. It is surprising, however, that, although *deserens* and *hdecipiens* (producing *rubrinervis* and *erythrina*, respectively) have arisen on numerous occasions from DE VRIES' *lamarckiana*, other complexes with the *hdecipiens* end arrangement have arisen only rarely. This might suggest that the lethals are so placed in *lamarckiana* that there are not very many ways available for the production of such a complex. Possibily we should consider that there is a greater likelihood of the *velans* lethal being in segment 13, let us say, than in segments 5 or 6. The fact that *hblandina*, which is another alethal complex derived from *lamarckiana*, but with a different end arrangement, lacks 13·14 would be in line with this supposition.

The position of velans and gaudens lethals

Little is known with regard to the position of the zygotic lethals forming the balanced lethal system in *Oe. lamarckiana*. CATCHESIDE (1936) is inclined to believe that the balanced lethal mechanism is not composed of a single lethal gene in each complex, but that "there is a vital chromosome balance involved, determined by the disposition of deficiencies and reduplications within the complexes." If deficiencies sufficiently large to have a lethal effect are present in a number of chromosomes in each complex, this would mean that there should be a number of chromosomes in each complex incapable of existing in double dose; if, however, but a single lethal gene or deficiency exists in each complex, then there should be but one chromosome in each complex incapable of existing in homozygous condition.

From our knowledge of the segmental arrangements of the complexes present in *erythrina*, it is clear that *hdecipiens* has a minimum of three chromosomes (and hence six ends) which have definitely been derived from the *velans* complex and which are capable of existing in double dose, since they form pairs with the corresponding chromosomes in the unaltered *velans* complex in *erythrina*. These chromosomes are 3·4, 9·10, and 11·12. It is obvious that these ends, at least, do not carry zygotic lethals. We have shown above, however, that ends 13 and 14 may actually be derived from *gaudens*, and ends 5 and 6 have definitely come from *gaudens*. We are justified, therefore, in assuming that *velans* lethals are absent from chromosomes 3·4, 9·10, and 11·12; which of the remaining ends of *velans* carry lethals cannot be ascertained at present, since we do not know which one of the 24 possible sequences of interchange listed in the table has led to the production of *hdecipiens*. We may say this much, however, that no matter how *hdecipiens* has arisen, ends 5 and 6 can theoretically carry *velans* lethals, and ends 7, 8, 13, and 14 still remain as possible positions for them. With

regard to *gaudens*, we may say that its lethals cannot reside in 5·6, but there is a theoretical possibility of their presence in any or all of the other *gaudens* chromosomes found in the circle of 12 of *lamarckiana*.

Phylogenetic significance

Two facts of phylogenetic importance are revealed by the present study. (1) Similarity in segmental arrangement does not always mean close phylogenetic relationship. *ʰDecipiens* appears to differ from "*hookeri*" by a single interchange, yet it is obvious that it is not removed from *ʰhookeri* by a single interchange. It arose by at least two interchanges from *velans*, which in turn is at least one interchange removed from *ʰhookeri*. Hence, *ʰdecipiens* is at least three interchanges removed from *ʰhookeri* and owes its apparent cytological resemblance to the latter complex to convergence. If interchanges occur more or less at random between the various ends, and there is reason to think that this may be the case, the probabilities are against the occurrence of convergence, so that we are inclined to consider the case of *ʰdecipiens* an exceptional one and to hold that, in general, similarity of segmental arrangement is an indication of close phylogenetic relationship. The relationship of *ʰdecipiens* and *ʰhookeri*, however, must serve to emphasize the need for caution in interpreting data obtained from the comparative study of wild races.

(2) Even if we can assume that two complexes are separated from each other by a given number of interchanges, it is still true that these complexes may bear any one of a number of theoretically possible relationships to each other. If we assume that *velans* and *ʰdecipiens* are actually removed from each other by two interchanges, as they seem to be, this still does not tell us which two of many possible interchanges have occurred in the formation of the newer complex from the older. The newer complex could have arisen simply by two successive interchanges between chromosomes of the older complex from which it appears to differ by two interchanges. It could have come about, however, in other ways as follows: (1) by an initial interchange involving a chromosome from each of the complexes present in the parent strain or (2) by an initial interchange between two chromosomes, both of which belonged to the complex opposed to the one from which the newer complex seems to be descended segmentally, any of these initial interchanges being followed by a second interchange involving (a) two chromosomes from one or the other of the original complexes or (b) one chromosome from each of the original complexes.

With this situation clearly in mind, and considering all the evidence from other sources, we apparently are justified in adopting the following working hypothesis:

(1) The number of interchanges by which two complexes appear to differ may or may not represent the true measure of cytological divergence between these complexes. When this number is small, the chances are strong that it actually represents the number of interchanges separating them, but when the number becomes larger, the chances of this become rapidly reduced.

(2) Even if complexes are actually separated by the number of interchanges by which they appear to differ, it does not follow necessarily that this represents a measure of the amount of genetical divergence. After one or two interchanges have occurred, the newer complex may have identically the same genes which one of the complexes in the parent plant had (if the interchanges have involved only chromosomes of this one complex).[7,8] On the other hand, the newer complex may have chromosome segments, and hence genes, belonging to both of the opposing complexes in the parent plant, and so it may have departed widely in genetical constitution from either of these complexes. Thus, *[h]decipiens* seems superficially to be descended on the cytological side from *velans* and to differ from it by two interchanges, but genetically, it possesses *gaudens* genes in a proportion which cannot as yet be ascertained. The same situation may apply in the case of the complexes found in wild races. Complexes in different races may seem to differ by one or two interchanges from each other, and this may represent the true measure of their cytological divergence. They may or may not be closely related genetically.

(3) This fact, however, does not mean that resemblance in segmental arrangement has lost its phylogenetic significance. *[h]Decipiens* may have fewer *gaudens* genes or more of them—it may even be predominantly *gaudens*, and yet there can be no question but that *velans* is in its line of ancestry. It may not stem entirely from the latter, but it does derive at least a part of its constitution from this complex. Two complexes in the wild also may show a close resemblance in segmental arrangement. They may have many (or most) genes in common, or they may be quite diverse genetically. In either case, a close resemblance in segmental arrangement is probably significant phylogenetically.

This fact will explain many cases which might otherwise be difficult of explanation. For instance, the *a* complexes of the *biennis* group have so far been found to produce phenotypic characters of the *biennis* type, very different from the phenotypes shown by the *hookeri* alliance. Nevertheless, they show resemblances to the *hookeris* in segmental arrangement. In the possibilities outlined above we have a possible explanation of this situation.

[7] This complex could not combine with its ancestral complex if the latter possessed a zygote lethal.

[8] The possible occurrence of position effect is not considered here.

SUMMARY

Oenothera lamarckiana habitually throws a small percentage of "half-mutants," of which *erythrina* is one. This form is made up of two complexes, one of which is the unmodified *velans* of *lamarckiana*, the other a modified complex called *ʰdecipiens* which appears to have derived some of its genetical make-up from both *velans* and *gaudens* of *lamarckiana*. A comparison of these three complexes from the standpoint of segmental arrangement suggests that *ʰdecipiens* has been derived from *velans* by two interchanges, but the apparent presence of *gaudens* genes in *ʰdecipiens* makes this supposition unlikely. In this paper it is shown that there are 24 ways by which a complex with the *ʰdecipiens* segmental arrangement could have arisen from the complexes of *lamarckiana*, assuming a succession of two interchanges. In each of these, *gaudens* chromosomes are involved in one or the other of the interchanges, and in some cases only *gaudens* chromosomes are involved in both interchanges. In all cases, however, the resultant "*ʰdecipiens*" has a mixture of *velans* and *gaudens* segments. However, not all of the 24 ways here listed are necessarily actual possibilities, the actual number of ways in which the *ʰdecipiens* segmental arrangement could be formed being determined by the position of *velans* and *gaudens* lethals in their respective complements.

The *velans* lethals cannot be present in ends ·3, ·4, ·9, ·10, ·12, and the *gaudens* lethals are not in ·5 or ·6.

The fact that *ʰdecipiens* seems to differ from *ʰhookeri* by a single interchange but is known to have been derived from *lamarckiana* by at least two interchanges emphasizes the fact that similarity in segmental arrangement between complexes is not always an exact indication of the degree of phylogenetic relationship between these complexes. One must exercise proper caution in ascribing to similarity in segmental arrangement its proper phylogenetic significance.

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GENETIC EFFECTS OF ULTRAVIOLET RADIATION IN MAIZE. IV. COMPARISON OF MONOCHROMATIC RADIATIONS[1]

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INTRODUCTION

EARLIER investigations with various heterochromatic radiations (STADLER and SPRAGUE 1936b, c) indicated that λ3131 Å and longer wave lengths were genetically ineffective or nearly so, while λ3022 and shorter wave lengths were effective but with pronounced differences in efficiency.

The measure of genetic effectiveness in these experiments was the frequency of endosperm deficiencies. Since these deficiencies may be identified in the seeds which develop directly from the treated pollen, they provide a convenient measure of genetic effectiveness and make it possible to compare wave lengths and doses on the extensive scale required for significant quantitative comparisons.

The high frequency of coincidence of genetically independent mutations (STADLER and SPRAGUE 1936a) suggested that, although the pollen grains were in a single layer during irradiation, there were considerable differences in the dose applied to the gametic nuclei in the different pollen grains treated. Such differences would be expected if the radiation is highly absorbed in the overlying cytoplasm, since the gametic nuclei are eccentrically located and casual orientation would place them at different levels in different pollen grains.

This heterogeneity of the treated sample, owing to variation in internal filtration among the pollen grains, could result in a serious error in the determination of relative effects of different wave lengths or of different doses at the same wave length. In an investigation of the frequency of occurrence of deficiency for a specific region, for example, it would tend to reduce the relative frequency of effects of larger doses, for the probability of affecting pollen grains with the more favorably oriented nuclei would be greatest for the first unit of dosage and, as these individuals were affected, the probability of successful hits in later increments of dosage would become steadily smaller. The resulting "levelling" of the dosage curve would be expected to vary in amount at different wave lengths, depending upon the rate at which the radiation is absorbed in the overlying material. A

[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. DEPARTMENT OF AGRICULTURE, and Departments of Field Crops and Physics, UNIVERSITY OF MISSOURI. Missouri Experiment Station Journal Series No. 763.

TABLE 1

Preliminary comparisons of embryo abortion and endosperm deficiency produced by monochromatic ultraviolet radiation. (Stadler and Uber 1938)

WAVE LENGTH Å	DOSE IN 10^3 ERGS/MM^2	EMBRYO ABORTION		ENDOSPERM DEFICIENCY (TOTAL FOR *A, Pr, Su, Wx*)		
		NUMBER TESTED	NUMBER ABORTED	NUMBER TESTED	DEFICIENT ENDOSPERMS	
					NUMBER	%±S.E.
2352	1.0	530	5	468	11	2.4±0.7
	2.1	429	0	377	22	5.8±1.2
2483	0.6	584	2	417	14	3.4±0.9
	4.0	165	3	144	16	11.1±2.6
2652	0.9	646	12	625	49	7.8±1.1
	1.9	255	7	223	27	12.1±2.2
	2.8	264	8	231	32	13.9±2.3
	6.2	255	19	223	42	18.8±2.6
	12	81	15	71	13	18.3±4.6
2804	0.7	791	3	663	40	6.0±0.9
	1.5	293	3	257	27	10.5±1.9
	2.1	315	9	262	33	12.6±2.1
	4.5	332	31	252	54	21.4±2.6
	9.0	168	7	147	49	33.3±3.9
2967	0.8	105	0	92	1	1.1±1.1
	1.7	290	0	272	10	3.7±1.1
	2.5	253	1	222	22	9.9±2.0
	5.5	622	8	500	119	23.8±1.9
	11	158	2	138	47	34.1±4.0
3022	2.6	321	2	281	3	1.1±0.6
	5.9	92	2	88	5	5.7±2.5
	57	266	8	228	56	24.6±2.9
3131	2.7	331	3	290	2	0.7±0.5
	5.9	100	0	100	0	0
	60	490	3	399	3	0.8±0.4
	600	440	2	392	45	11.5±1.6
Control		614	2	536	6	1.1±0.5

comparison of the relative effectiveness of different wave lengths, at doses equal in the energy incident at the surface of the pollen grain, therefore might yield different results when different doses were used as the basis of comparison.

Preliminary comparisons of monochromatic radiations were made in 1937 (STADLER and UBER 1938). Since the experimental data were not included in the published abstract, they are now presented in table 1.

These trials, while showing large differences in the effect of different wave lengths upon the frequency of endosperm deficiency, indicated also that the dosage curves at the different wave lengths were widely different. A comparison of the deficiency rates at the smallest doses used shows that the most effective wave lengths were λ2652 and λ2804, with lowered effectiveness on the short wave length side of this region and much lowered effectiveness on the long wave length side. λ3131 was ineffective at doses comparable to those used for the other wave lengths, but by using a very much larger dose tolerated at this wave length, it was possible to produce appreciable effects.

It was evident from these trials that the determination of wave length effectiveness would require closer study of the dosage relation and of internal filtration in the pollen grain. Spectrophotometric studies of absorption in the pollen grain wall and contents were made (UBER 1939), and a more extensive determination of genetic effects was undertaken. These experiments were carried on in 1938–1940 and are the subject of this report.

REVIEW OF LITERATURE

This brief review of relevant papers is limited to researches dealing with genetic effects of monochromatic radiations.

NOETHLING and STUBBE (1934, 1936; see also STUBBE and NOETHLING 1936) determined the frequency of mutation in *Antirrhinum majus* following exposure of the pollen to various monochromatic radiations. Since the occurrence of mutation in each treated pollen grain must be determined by segregation of the mutant in an F_2 culture, the numbers involved in individual comparisons were necessarily limited. An extensive study was made, involving mutation tests for more than 3000 treated pollen grains, and the identification of more than 100 mutations. The spontaneous mutation rate in the standard line used was 1.6 percent. Significant increases in mutation frequency were obtained by treatment with λ2652, λ2967, λ3022, and λ3131, the highest rates observed being about four times the control frequency.

Several doses were compared at λ2652 and λ2967. At λ2652 doses of 2 M (where M represents 10^3 ergs/mm^2) failed to increase the mutation rate significantly. Larger doses resulted in significant increases in mutation frequency, but the mutation rate increased very slowly with increase in dose. For example, at a dose of 2.7 M the mutation rate was approximately twice that of the control; at doses of 14.6 and 50.3 M it was about three times the control rate. At λ2967 all four doses applied yielded significant increases over the spontaneous mutation rate, but though the doses covered a wide range (1.8 M–65.0 M), there were no significant differences in the mutation

rates observed. λ3131 at a dose of 120 M approximately quadrupled the spontaneous rate, as did λ3022 at a dose of 65 M.

From these results NOETHLING and STUBBE conclude that λ2967 is more effective than λ2652 or λ3131 and that the maximum of genetic effectiveness is at approximately 3000 Å. The transmission of ultraviolet radiation through a pollen suspension in 30 percent alcohol showed a minimum at 3000 Å and was considered to be in good agreement with the data on genetic effectiveness.

KNAPP, REUSS, RISSE, and SCHREIBER (1939) compared the effectiveness of six ultraviolet wave lengths, ranging from λ2536 to λ3131, in inducing mutation in *Sphaerocarpus Donnellii*. This material is especially favorable since the radiation may be applied to the spermatozoid, which is an almost naked nucleus about 0.5μ in thickness. The spermatozoids were irradiated in water suspension, and the mutations were identified by tetrad analysis of the sporogonia of F_1 individuals. The technic has been described by KNAPP (1937). By the analysis of 50 to 75 individuals from each treatment, it was possible to show sharp differences in genetic effectiveness of the wave lengths applied. A uniform dose of 2×10^3 ergs/mm^2 was used. No mutations were found in the control or in the series irradiated at 3131 Å; a few occurred at 3022 Å and 2967 Å; but mutations were frequent at 2804 Å, 2652 Å; and 2536 Å. The highest frequency occurred at 2652 Å, although the significance of the differences between the mutation frequencies observed at the shorter wave lengths is perhaps questionable.

EMMONS and HOLLAENDER (1939) have investigated the effectiveness of monochromatic radiation in inducing cultural variations in the fungus *Trichophyton mentagrophytes*. Such variations occur frequently without treatment in old cultures but are not found in young cultures. By the application of ultraviolet radiation or various other treatments, variants may be induced in young cultures. The variants, whether spontaneous or induced, are permanent through numerous transfers. Unfortunately the fungus has no known sexual stage, and it is therefore impossible to investigate the genetic nature of the variants. The treatments were applied to spores in suspension. Wave lengths differed in lethal effect and in the frequency of induced variants. With increasing dose, the percentage of variants among the surviving individuals rose to a maximum and then declined. This is a phenomenon not previously found in genetic experiments with ultraviolet radiation and one which suggests the possibility that the induced changes involved in the production of variants and in the production of lethal effects are directly related. In general, λ2536 and λ2652 were most effective in killing and in the production of variants. λ2950 was distinctly less effective than shorter wave lengths.

EXPERIMENTAL TECHNIC

Apparatus

The mercury discharge tube, to which several references are made later, was a low-pressure type (Hanovia Sc-2537) operating at 5000 volts and 100 milliamperes. It emitted primarily the 2536 Å line of mercury (STADLER and SPRAGUE 1936c) and was used without any filter except a quartz microscope slide which covered the pollen.

For the greater part of the pollen treatments, monochromatic radiation was obtained with the aid of a large crystal quartz monochromator, described in detail elsewhere by UBER and JACOBSOHN (1938), which was designed specifically for the irradiation of pollen. To this end, it had a vertical disposition so that the pollen to be treated would lie on the horizontal surfaces provided by trays (2 mm. × 50 mm. in area) at each ultraviolet emission line of the mercury arc. The arrangement of the individual trays along the spectrum was such that any tray could be inserted or removed independently of the others.

The mercury arc source for the monochromator was of the water-cooled capillary type and operated at atmospheric pressure. Its design and characteristic features have been discussed elsewhere (UBER 1940). Since the ultraviolet energy output of an arc would usually decrease to about one-half of its initial value after three hours of continuous operation, it was customary to replace it with a new or reconditioned one at that point. An experimental check on the relative intensity of λ2536 compared to λ3131 did not reveal any appreciable variation due to the decline in output of the arc over the time interval indicated.

The surface-type vacuum thermopile used to measure the radiation was calibrated with a standard carbon-filament lamp (C-241) obtained from the U. S. Bureau of Standards. The radiation has been measured in ergs per square millimeter of the treatment surface, but for convenience in presentation, the dose is usually stated in M units where 1 M represents 10^3 ergs/mm^2.

Physical Measurements

For the purpose of measuring the dosage rate during treatment, a slot beneath the λ3131 tray permitted the radiation to pass through to the thermopile. All doses were based on energy measurements at λ3131, the values for other lines being computed from relative intensity data. Table 2 gives the wave lengths employed, their relative intensities with respect to λ3131, and average values for the dosage rates in ergs/mm^2 per minute. The lengths of time required for administering the various doses varied from around 15 seconds up to 30 minutes. In order to make the exposure times susceptible to accurate measurement for the smallest doses at some

of the lines, an iris diaphragm was placed in the collimating tube of the monochromator; in that case the dosage rate was reduced to about 12 percent of the values shown in Table 2.

Thermopile readings were taken at ten to 15 minute intervals as a basis for calculation of the doses actually applied. In making the treatments, the time required for the desired dosage was estimated from the previous intensity readings, and the dose actually applied in each treatment was calculated later from the completed intensity curve. The treatments aver-

TABLE 2

Characteristics of the monochromator radiation.

λ	INTENSITY RELATIVE TO λ3131	AVERAGE DOSAGE RATE	IMPURITY OF OTHER LINES AT λ3131	
			WITHOUT FILTER	WITH FILTER*
Å		ergs/mm²/minute	Percent	Percent
3131	1.000	30,000		
3022	.506	15,000	0.100	.0210
2967	.256	7,500	.050	<.0074
2925	.033	990	.003	trace
2894	.086	2,580	.020	
2804	.167	5,010	.030	
2752	.071	2,130	.015	
2699	.065	1,950	.008	
2652	.351	10,530	.050	
2536	.274	8,220	.040	
2483	.103	3,090	.010	
2399			.003	
2378	.0487	1,460	.003	
2352			.001	
			0.333	<0.025

* Corning #014, clear blue fluorescing glass filter, one mm thick.

aged in a single figure therefore may include doses varying as much as ten percent from the average. The doses as stated in the tables are the unweighted mean of the actual doses applied in the treatments averaged.

In practice, the uniformity of the ultraviolet illumination over the area of a spectral line covered by the tray was checked visually with a fluorescent screen while aligning the arc source. Tests of the uniformity made with a Geiger-Mueller photoelectric counter showed that in going 1.75 cm on either side of the center along the five cm trays, there was a reduction in intensity of three percent compared to the intensity at the center; at the ends of the trays, the corresponding reduction in intensity was ten percent.

Purity of the radiation is an important factor, especially at the longer

wave lengths which are relatively ineffective genetically. By analyzing the radiation photographically with the aid of a medium Hilger quartz spectrograph and suitable filters, approximate estimates of spectral impurity have been obtained. These values for λ3131 are given in the fourth column of table 2 and include only the ultraviolet impurities of wave lengths less than 3131 Å, longer radiation not being appreciably effective genetically. It will be noted that the measured impurity at λ3131 owing to the radiation of all other important mercury lines in the ultraviolet region is around one-third of one percent. To a rough approximation, the percentage of impurity at the other wave lengths is inversely proportional to their relative intensity. At λ2652, for example, which is about one-third as intense as λ3131, the total impurity would be about one percent. For very weak lines, the impurity may become a serious factor.

The total impurity at λ3131 could be reduced to less than one part in 4,000 by the use of a short wave length cut-off filter consisting of a one mm thick Corning #014 glass plate, whose transmission at λ3131 was 58 percent.

Treatment Routine

The pollen was irradiated in an insulated field house provided with mechanical refrigeration. Thus the treatment room could be air-conditioned to the extent that the temperature remained within the range 20–23°C, and the relative humidity varied from 45–60 percent. These conditions contrasted with outside temperatures as high as 37°C and with much lower relative humidity readings.

The treatments were made in the morning beginning as early as pollen-shedding conditions would permit (6:30 to 8:30 a.m.) and continued until about noon. Tassel branches bearing mature anthers were removed from the parent plant when the anthers began to dehisce, wrapped in moist paper towelling, and held in the treatment room for use as needed. The pollen was poured directly from the anther to the slide for treatment, the spheroidal grains readily rolling into place in a single layer.

No accurate comparisons to determine killing effects of treatments have been made, but large differences in tolerance for the wave lengths compared are obvious. In general the longer wave lengths were tolerated at much larger doses than the shorter. In spite of the uniform conditions of temperature and humidity during treatment, there was a pronounced loss of tolerance when the weather became hot and dry. This might have been due to changes in the condition of the pollen at shedding or in the conditions of pollen germination or pollen tube growth. The most favorable period for treatment at Columbia is the early part of the pollen-shedding season (about June 20 to July 5). Under optimum weather conditions, the maxi-

mum tolerated dose at the shortest wave lengths (2352–2399 Å) was about 2 M. At λ2483–λ2804 much larger doses were tolerated, and a small set of seed was sometimes obtained with treatments of 16–32 M. At λ2967 under similar conditions a dose of 64 M could be applied, and at λ3022 a dose of 256 M. At λ3131 doses of more than 1,000 M have been applied without apparent injury to the pollen. With less favorable weather, tolerance was reduced, but relative tolerance at the different wave lengths was not greatly changed. Under the hot dry conditions characteristic of late July and early August in this region, tolerance is so greatly reduced that experiments with ultraviolet treatment are not practicable.

As in previous experiments, the general method followed was the pollination of a multiple recessive stock by a multiple dominant. Since fractional endosperm deficiencies for the genes *Y* and *Wx* cannot be detected with certainty in all cases when the aleurone is colored, the comparisons in this study were based entirely on deficiencies for the genes *A* (colored vs. colorless aleurone), *Pr* (purple vs. red aleurone), and *Su* (smooth vs. wrinkled endosperm).

The seed parent stock (*R*^{g} *C a pr su y wx b pl*) was grown from several closely related ears, with successive plantings so as to provide a supply of ears through the period of favorable weather. The pollen parent stock was an inbred strain dominant for the genes mentioned. The pollen used for each day's treatment was obtained from a single plant, and the same plants were used so far as possible for treatment on successive days.

Classification of Effects

The effects recorded for the treatments compared in the study are "endosperm deficiencies" and "embryo abortions." The term endosperm deficiency is applied to all losses of effect of the dominant marker genes *A*, *Pr*, and *Su*. It is possible that some of these losses result from mutation rather than deficiency, but comparable trials with the linked genes *C*. *Sh*, and *Wx* indicate that the great majority if not all of the cases represent the loss of a chromosome segment.

In earlier experiments each fractional deficiency was classified as to the area involved. Since the proportion of fractionals and the distribution of fractionals in the various size classes in the earlier experiments were found to be independent of dosage and wave length, the deficiencies in the present experiment have been classed merely as "entire" and "fractional." As previously reported, the most frequent type of fractional is that in which the deficient sector includes about one-half of the endosperm.

The subjective element in the determination of the frequency of genetic alterations is at a minimum in the case of these endosperm deficiencies, since the three dominant markers used are usually quite clear in effect.

The loss of *A* is always unmistakable, and in the stocks used *Su*-loss is almost equally distinct. In occasional ears the difference due to *Pr*-loss is faint in some seeds. A few such ears occurred in the present experiment, and in these the *Pr* data are excluded and the number of seeds examined is taken as $\frac{2}{3}$ of the actual number. Since the average frequency of *Pr* loss is lower than that of *A* loss, this introduces a slight error in comparisons. Only 15 of the 369 ears examined in the experiment were affected in this way. Since these were distributed at random among the treatments compared, the error is negligible.

Loss of *Pr* cannot be detected in a seed or sector which is also deficient for *A*. A fractional deficiency for *A* would be recognizable in a seed entirely deficient for *Pr*, but the resulting endosperm would have one sector colorless and the other red, a type which could be produced also by a fractional deficiency for *A* with a complementary fractional deficiency for *Pr*. It was noted that in the majority of endosperms with complementary colorless and red sectors, the aleurone cells along the margin separating the sectors were purple, while in other endosperms of this class there was no appearance of purple pigmentation in the marginal cells. The ratio of the two classes was approximately the same as the ratio of fractional to entire *Pr* deficiencies among the seeds without deficiency of *A*. It is therefore assumed that the seeds of the first class described represent fractional deficiencies for both *Pr* and *A*, while those of the second class represent entire deficiencies for *Pr* with fractional deficiency for *A*. Presumably the development of the purple phenotype (*A Pr*) occurs in the marginal cells as a result of the interaction of adjacent cells of *A pr* and *a Pr*.

Coincident losses of *A* and *Su* or of *Pr* and *Su* are detectable in all combinations. The coincidences observed include cases of entire deficiency for both markers concerned, cases of entire deficiency for one with fractional deficiency for the other, and cases of fractional deficiency for both, which may involve the same sector or different sectors. In almost all cases, when two deficient sectors occur in the same seed, the two together cover the entire endosperm, even though the size of the two sectors may be very different. This supports the view that the fractionals of the size classes larger or smaller than "$\frac{1}{2}$" are in the main the result of unequal development of sectors, and that the fractional deficiencies in general represent endosperms which at a two-cell stage had one deficient and one non-deficient cell.

Coincident loss of *A* and *Su*, or of all three markers, involving the entire endosperm, results in a colorless, sugary endosperm which is phenotypically indistinguishable from an endosperm produced by pollen contamination from the multiple recessive stock. Although the seed parent cultures were regularly detasseled before pollination, there is always a possibility of occasional contamination from neighboring plots or from tassels

or florets missed in detasseling. Genetic tests of several seeds of this type were made, and some were found to be the result of pollen contamination. All seeds of this type have therefore been excluded from the data. This may result in the exclusion of some cases of induced deficiency, and the error might affect disproportionately the more effective treatments. However the total number of seeds excluded as possible contaminations was only 17, among a total of 35,086 seeds examined and 2,775 deficiencies identified.

The number of coincident deficiencies included in any of the deficiency counts may be determined from the tables by comparison of the data given for "number of deficient seeds" and "number of deficiencies."

The term "embryo abortion" is applied to those seeds in which a normally developed embryo is not produced. In external appearance the seed seems to lack an embryo, although usually the endosperm is fully developed. On dissection it is often possible to find dead rudiments of the embryo. This is the type of seed usually designated as "germless."

A small proportion of germless seeds is found in many varieties of maize, and though they are more common in some strains than in others, there is no strain known to us in which a germless seed is not occasionally found. Among inbred strains distinct differences in the frequency of germless seeds appear, but according to WENTZ (1930) the inheritance of these differences is usually complex. In a few cases simple recessive factors for germless seed have been identified (DEMEREC 1923; WENTZ 1930; see also EMERSON, BEADLE and FRASER 1935). The frequency of occurrence of germless seeds is markedly increased by X-ray or ultraviolet treatment of pollen.

EXPERIMENTAL RESULTS

Control

Control pollinations were included among the treatments made each day and were distributed at random among the cultures of the female parent stock. The control pollen was handled like the treated pollen except for the application of ultraviolet radiation, some samples being held 15 seconds and others three minutes to correspond to the approximate length of time required for the treatments used. In addition a special contro was included for comparison with λ3131 treatments, since these treatments required an exposure of 20 to 30 minutes. Each treatment at λ3131 was paralleled by a control held on a pollen slide for an equal period of time.

The frequency of endosperm deficiency in the two control populations was not significantly different. The results are shown in table 3.

There was no indication of heterogeneity among the ears included. Two large ears had three deficiencies each, three ears had two deficiencies each,

and the remaining 20 deficiencies occurred singly. The distribution of deficiencies among the multiple recessive cultures was random.

The total control population included 68 seeds with aborted embryos, or 0.93 ±0.11 percent. It was obvious, however, that the population was not homogeneous, for six of the 57 ears had four or more aborted embryos each, and one of these had 13 aborted embryos in a total of only 88 seeds. Four of these six ears occurred in one culture (23-221) of the female parent stock. The control pollinations were distributed at random among the cultures of the female parent, and there were only three cultures in which eight or more control ears were included. In culture 23-221 each of nine control ears had one or more aborted embryos, and the total frequency for the culture was 36/1251 (2.88 percent). In culture 23-201 there was only one aborted embryo among the eight ears tested. In culture 23-203 there were ten control ears, among which six had one or more embryo abortions each, the total frequency for the culture being 13/1741, or 0.75 percent.

TABLE 3

Frequency of endosperm deficiency in control pollinations.

CONTROL STOCK	NUMBER EARS	NUMBER DEFICIENT SEEDS	ENDOSPERM DEFICIENCIES						TOTAL FREQUENCIES	
			A		*Su*		*Pr*			
			E*	F*	E	F	E	F	NUMBER	%±S.E.
General control	45	27	0	13	4	0	0	10	27/5949	0.45±0.09
Special control for λ3131	12	5	0	2	0	0	0	3	5/1459	0.34±0.14
Total	57	32	0	15	4	0	0	13	32/7408	0.43±0.07

* E=entire; F=fractional

This comparison shows that embryo abortion, independent of the pollen treatment, is distinctly more likely to occur in some of the female stock cultures than in others. In plants with the tendency toward frequent embryo abortion, the average frequency was less than three percent, far below the frequency induced by heavy treatment of pollen. But among such plants wide fluctuations occur in frequency of abortion. Obviously in comparisons based on a small number of ears, the standard errors calculated from the totalled populations would not accurately measure the true sampling error

It is desirable therefore to limit the comparisons of treatments in their effect on the frequency of embryo abortion to the ears of cultures free from this tendency. The number of control ears included in each culture was not sufficient in all cases to determine the presence or absence of the abortion tendency. A further comparison of the female stock cultures was there-

fore made in pollinations with very small doses or doses with little genetic effect. For this purpose treatments of ½ M or less at λ2894, λ2925, λ2967, treatments of 2 M or less at λ3022, and a treatment of 500 M at λ3131 were used. On the basis of this comparison several cultures of the seed parent stock were found to include plants with the tendency toward embryo abortion. Comparison of the effects of the treatments upon the frequency of embryo abortion has therefore been made only from the pollinations on ears of the remaining cultures. In these cultures the spontaneous frequency of germless seeds was 0.31 percent. It is possible, however, that some of these cultures may have included individuals with the tendency toward embryo abortion.

The foregoing description applies to the control of the extensive trial of monochromatic radiations made in June and July, 1938. Additional comparisons made in 1939 and 1940, chiefly with the discharge tube radiation, were accompanied by separate controls, which are reported with the data of the experiments concerned.

The Dosage Relation

Extensive dosage comparisons were made at λ2652 and λ2967, since the preliminary trials made in 1937 had indicated extreme levelling of the dosage curve at the former wave length and much less levelling at the latter. At λ2652 the doses applied ranged from approximately ¼ M to 16 M; at λ2967, from approximately 2 M to 64 M. At each wave length, larger doses also were applied, but the yield of seeds was too small to permit comparison.

The results are shown in tables 4 and 5. The significance of the comparisons in frequency of embryo abortion is limited because of possible fluctuation in spontaneous frequency, as has been mentioned. There is, however, a distinct effect of the treatment upon the frequency of embryo abortion,

TABLE 4

Relation of dosage at λ2652 to frequency of embryo abortion and endosperm deficiency.

DOSE (M)	NUMBER EARS	FREQUENCIES OF EMBRYO ABORTION		NUMBER DEFICIENT SEEDS	ENDOSPERM DEFICIENCIES						TOTAL FREQUENCIES	
					A		*Su*		*Pr*			
		NUMBERS	%±S.E.		E*	F*	E	F	E	F	NUMBERS	%±S.E.
.24	16	18/1880	1.0±0.2	26	3	16	0	3	2	2	26/2047	1.3±0.3
.49	10	6/1006	0.6±0.2	16	2	4	2	4	1	3	16/1311	1.2±0.3
.99	7	8/932	0.9±0.3	40	4	19	4	5	0	12	44/989	4.4±0.7
1.99	9	23/1107	2.1±0.4	102	9	47	8	25	6	28	123/1063	11.6±1.0
3.87	4	7/262	2.7±1.0	29	1	18	1	4	2	6	32/307	10.4±1.7
7.85	9	70/927	7.6±0.9	124	12	62	7	29	4	27	141/927	15.2±1.2
15.6	5	20/106	18.9±3.8	16	4	6	2	2	2	3	15/106	17.9±3.7

* E = entire; F = fractional

TABLE 5

Relation of dosage at λ2967 to frequency of embryo abortion and endosperm deficiency.

DOSE (M)	NUMBER EARS	FREQUENCIES OF EMBRYO ABORTION		NUMBER DEFICIENT SEEDS	ENDOSPERM DEFICIENCIES							
					A		*Su*		*Pr*		TOTAL FREQUENCIES	
		NUMBERS	%± S.E.		E*	F*	E	F	E	F	NUMBERS	%± S.E.
1.99	11	5/1006	0.5±0.2	67	3	33	4	12	3	15	70/1498	4.7±0.5
3.98	6	8/658	1.2±0.4	67	4	31	1	13	2	20	71/1160	6.1±0.7
7.69	6	16/684	2.3±0.6	74	6	34	3	12	7	16	78/684	11.4±1.2
15.6	6	4/131	3.1±1.5	170	15	83	7	39	9	33	186/671	27.7±1.7
32.9	6	22/283	7.8±1.6	76	9	37	3	19	3	15	86/283	30.4±2.7
38.6	5	14/118	11.9±3.0	32	7	15	4	4	2	5	37/120	30.8±4.2
46.4	5	7/160	4.4±1.6	59	10	33	5	4	1	8	61/198	30.8±3.3
53.7	3	2/46	4.4±3.0	32	6	16	0	8	2	6	38/98	38.8±4.9
65.6	5	14/134	10.5±2.6	56	4	21	6	22	5	12	70/142	49.3±4.2

* E = Entire; F = fractional

which results, at least at the larger doses, in frequencies clearly beyond the maximum spontaneous frequency. At λ2652 the increased frequency of embryo abortion is evident at doses of 2 M and higher, and the frequency rises steadily with the dose. At λ2967, at the smaller doses used, the frequency of embryo abortion is much less than at equal doses of λ2652. Even at a dose of 16 M, λ2967, although it yields many more deficiencies than λ2652, yields only about one-sixth as many aborted embryos. Nevertheless it is clear that λ2967, at the largest doses tolerated, has a significant effect upon the frequency of embryo abortion.

The course of the dosage curve for endosperm deficiency is distinctly different for the two wave lengths compared. At λ2652 with the smallest doses used, the yield of deficiencies in excess of the control frequency is very slight. With increasing dosage, the deficiency rate rapidly increases to about ten percent at 2 M, but increases of dose beyond this point produce relatively little increase in the frequency of deficiency, an eight-fold increase of dose resulting in less than a doubling of the deficiency rate. At λ2967 a dose of 2 M has much less effect than a similar dose at λ2652, but with increased dosage the deficiency rate rises almost proportionately up to a dose of approximately 16 M. Beyond this point there is little increase in the yield of deficiencies with increasing dosage. A comparison of the two wave lengths at a dosage of 2 M indicates that λ2652 is the more effective, whereas at 16 M the reverse is true.

Effect of Size of Pollen Sample

Some deviation from a linear dosage relation might be expected to result from different conditions of competition at the different doses. The number of pollen grains applied is considerably in excess of the number of silks pollinated, but with increasing dosage the number of pollen grains capable

of accomplishing fertilization declines until with the largest practical doses only a few seeds are produced on each ear. There is therefore an opportunity for competition among equally treated but differentially affected pollen grains at the smaller doses, but because of the limited pollen specimens applied it would be minimized at the larger doses.

If injury affecting functional efficiency is correlated with genetic effect, the grains most affected genetically would be those at the greatest disadvantage in survival and in competition. Their lowered survival would reduce the frequency of deficiencies detected below the frequency actually induced, and among the surviving pollen grains competition would further modify the result to a degree dependent upon the quantity of functional pollen remaining. *A priori* it seems improbable that there would be much

TABLE 6

Relation of the quantity of pollen applied to the silks to the frequency of deficiencies. (Unfiltered radiation from mercury discharge tube, 7.5 minutes' exposure.)

POLLEN QUANTITY	NUMBER EARS	NUMBER DEFICIENT SEEDS	ENDOSPERM DEFICIENCIES							
			A		*Su*		*Pr*		TOTAL FREQUENCIES	
			E*	F*	E	F	E	F	NUMBERS	%±S.E.
1 anther	15	193	28	74	8	17	19	60	206/997	20.7±1.3
½ anther	16	121	13	62	3	16	10	32	136/687	19.8±1.5
¼ anther	15	86	9	37	7	8	8	25	94/434	21.7±2.0
Total	46	400	50	173	18	41	37	117	436/2118	20.6±0.9

* E=entire; F=fractional

correlation between genetic effect and injury to pollen function, for genetic effects are the result of alterations within the gametic nucleus while the effects on functioning are presumably due in the main to changes produced in other parts of the pollen grain.

If competition is a significant factor, the effect should be detectable in comparisons of treated pollen applied in varying quantities. A treatment dose with a distinct effect in reducing seed set was taken as a basis, and further reduction of seed set was obtained by reducing the size of the pollen sample.

In order to obtain adequate replication of the treatments, the discharge tube radiation was used. The pollen was irradiated for 7.5 minutes, a dose approximately equivalent to 8 M of λ2536. Eight samples were irradiated simultaneously in each treatment. In one treatment these consisted of approximately one anther each; in another treatment of one-half anther each; and in a third of one-quarter anther each. The three treatments were made consecutively, and on the following morning the experiment was

repeated. Two of the 48 ears were lost through earworm injury. The data obtained from the remaining ears are shown in table 6. Although the number of seeds per ear was considerably reduced by the reduction in quantity of pollen applied, no effect upon the deficiency rate is evident.

"Threshold Effect"

In the data for λ2652 (table 4) the course of the dosage curve suggests the possibility of a "threshold effect." Doses of ¼ M and ½ M had little if any effect upon the frequency of endosperm deficiencies, while there was a disproportionate increase at 1 M and 2 M. If the frequency of deficiencies in excess of the control at 1 M and 2 M is fairly representative of the effect of the treatment, the number of deficiencies found at ½ M is well below that expected on the basis of a linear dosage relation. While the deviation from linearity in this region of the curve is not certainly significant, there was additional support of the possibility in the results of comparisons of doses of ½ M and 2 M at many other wave lengths. The data available are shown in table 7.

TABLE 7

Relative effect of large and small dosages on the frequency of endosperm deficiencies at various wave lengths.

WAVE LENGTH Å	"2 M"				"½ M"				EXPECTED DEFICIENCIES ½ M
	DOSE	NUMBER EARS	FREQUENCY OF DEFICIENCIES		DOSE	NUMBER EARS	FREQUENCY OF DEFICIENCIES		
			NUMBERS	%± S.E.			NUMBERS	%± S.E.	
2925	2.00	3	46/510	9.0±1.3	0.47	6	5/619	0.8±0.4	15
2894	1.91	8	63/1010	6.2±0.8	0.50	11	22/1275	1.7±0.4	29
2804	1.97	7	94/1016	9.3±0.9	0.48	10	20/1749	1.1±0.2	45
2752	2.03	3	39/309	9.8±1.5	0.50	4	8/471	1.7±0.6	13
2699	1.96	2	21/268	7.8±1.6	0.48	5	10/569	1.8±0.6	13
2652	1.99	9	123/1063	11.6±1.0	0.49	10	16/1311	1.2±0.3	42
2536	1.96	7	124/802	15.5±1.3	0.49	12	34/1973	1.7±0.3	83
2483	1.96	9	98/987	9.9±1.0	0.49	12	41/2178	1.9±0.3	61
2378	2.04	2	16/231	6.9±1.7	0.48	4	8/635	1.3±0.5	12
Total			670/6852				164/10780		313

The numbers given under "Expected Deficiencies" are those which should occur if the effect of the lower treatment were directly proportional to that of the higher treatment, due allowance being made for the control frequency. The numbers observed at the lower doses are in general somewhat lower than the expected, and the total yield in excess of the control is less than half of that expected on the basis of a linear dosage relation.

A disproportionately low yield of deficiencies from small doses would be expected if deficiencies are produced by a multiple-hit mechanism, or if for any other reason a small dose may increase the probability of occur-

rence of deficiencies which it alone is not able to induce. At the larger doses, the levelling of the dosage curve expected as a result of variation in orientation of the pollen grains treated (cf. Introduction) might obscure the effect.

However, there is reason to suspect that, for differences of such small magnitude as those with which we are here concerned, the treatments at 2 M and $\frac{1}{2}$ M are not strictly comparable. There is at present no evidence that the frequency of deficiencies induced by a given dose of ultraviolet radiation is wholly independent of environmental conditions. In order to minimize the effects of such possible variations, an attempt was made in these experiments to seriate the treatments in such a way as to ensure the maximum comparability of treatments to be directly compared. For example, in the comparison of different wave lengths at a given dose, the treatments at the different wave lengths were made in rotation, the entire series being repeated as often as necessary to obtain the number of ears required. So far as possible dosage comparisons at the same wave length were seriated similarly, although the greater part of the available data on dosage effect comes from treatments seriated for wave length at constant dose rather than for dose at constant wave length.

In comparing doses of $\frac{1}{2}$ M with 2 M and larger doses, seriation for dosage was not feasible, since the very low dose of $\frac{1}{2}$ M required the use of the monochromator diaphragm to reduce the intensity of the radiation. The use of the diaphragm was not in itself the cause of the discrepancy, since comparisons of treatments with λ2652 at 2 M, made with and without the use of the diaphragm, showed no significant difference in frequency of induced deficiencies. The use of the diaphragm, however, made it necessary to apply the small doses in separate series of treatments. The data of table 7 for $\frac{1}{2}$ M represent the results of several series of treatments (with diaphragm) chiefly from June 23, 24, and 28, those for 2 M the result of separate series of treatments (without diaphragm) chiefly from June 22, 24, 25, and 27. The treatments with various wave lengths at $\frac{1}{2}$ M therefore are directly comparable with one another, but they are comparable with the treatments at 2 M only to the extent that the results are independent of the varying conditions at the different times of treatment.

Examination of the results summarized separately for each day of treatment indicated the possible occurrence of significant daily variation (see next section). A further trial of small doses of λ2652 was therefore made in 1940, with special precautions to avoid these and certain other possible sources of error. The plants pollinated were grown from a single selfed ear of an inbred *a C R* stock related to that used in the previous studies. The pollen parent in all pollinations was a single plant of an inbred dominant stock. This cross shows deficiencies only for *A*. The data therefore are free

from the possible slight errors due to variation of the sub-cultures of the parent stocks and to fluctuations in the phenotypic expression of *Pr* and *Su*. By the use of a commercial mercury arc with activated electrodes as a source, the variation of the radiation intensity with time was avoided, and better uniformity over the irradiated area was obtained. The treatments were made on July 2, 3, and 4; each day's run consisted of a continuous series with regular seriation of all doses and the control. The results are shown in table 8.

TABLE 8

Frequency of endosperm deficiencies (A) in relation to dosage (λ2652).

DOSAGE IN "M" UNITS	NUMBER EARS	FREQUENCY OF DEFICIENCIES	
		NUMBERS	%±S.E.
0.25	21	10/1524	0.7±0.2
0.50	20	27/2044	1.3±0.3
1.0	16	45/1907	2.4±0.4
2.0	10	37/690	5.4±0.9
Control	23	17/3172	0.5±0.1

Although the increase over the control frequency per unit of dose is again somewhat smaller at the smallest doses, the deviation from linearity is not statistically significant.

Daily Fluctuation

The extent of daily fluctuation is best determined from the results of treatments made with the discharge tube, since in these treatments usually four samples were treated simultaneously, and the data are therefore subject to a smaller sampling error. Treatments were made with a discharge tube in 1939 in several experiments in which the same male and female parent stocks were used as in the monochromator treatments. These experiments were concerned chiefly with the effect of size of the pollen sample and of irradiation from opposite directions, and their results are discussed in subsequent sections. They were not designed with the purpose of detecting daily variation, but since the comparisons made on different days included comparable treatments in many cases, the results will serve to indicate the amount of daily fluctuation. The unit of dosage was an exposure of seven and one-half minutes to radiation from the discharge tube at a distance of 21 centimeters above the pollen, with no filtration except that of a microscope slide of fused quartz covering the pollen (similar to that on which the pollen was placed). This is approximately equivalent to a dose of 8 M at λ2536. These treatments are comparable with the monochromator treatments except for the difference in the source of the radia-

tion and in the quantity of pollen treated. In the discharge tube treatments the pollen sample consisted of the pollen from three anthers of average size, instead of one as in the monochromator treatments.

During the period June 21 to July 1, treatments with the dose described above were included in trials made on six different days. On three other

TABLE 9

Daily variation in the frequencies of induced endosperm deficiencies.
(Discharge tube radiation, 7½ minutes, 21 cm.)

DATE (1939)	DIRECTION OF EXPOSURE*	POLLEN QUANTITY (ANTHERS)	NUMBER OF EARS	FREQUENCY OF DEFICIENCIES	
				NUMBERS	%±S.E.
June 21	A	3	2	38/169	22.5±3.2
June 22	B	3	2	35/154	22.7±3.4
June 23	A	3	4	136/531	
	B	3	4	82/309	
Total			8	218/840	26.0±1.5
June 24	A	3	4	102/411	
	B	3	4	80/332	
	A	1	4	36/152	
Total			12	218/895	24.4±1.4
June 26	A	3	4	98/581	
	B	3	3	95/392	
	B	1	4	54/251	
Total			11	247/1224	20.2±1.1
June 27	A	3	4	54/332	
	B	3	4	41/316	
	A	1	4	42/336	
Total			12	137/984	13.9±1.1
June 29	A	3	8	130/1004	12.9±1.1
June 30	A	1	8	115/496	23.2±1.9
July 1	A	1	7	91/501	18.2±1.7

* A=from above; B=from below

days of this period, though an identical treatment was not made, a treatment was included which was the same except that the radiation was applied from below instead of above the pollen, or that the treated specimen was reduced to the pollen from one anther. Since comparisons of consecutive treatments showed no significant difference due to the direction of exposure or to the pollen quantity, the results of these treatments also are included. The data are shown in table 9.

It is evident that the frequency of induced deficiencies at a given dose (or at any rate of surviving deficiencies) may differ very widely under the varying conditions of the different days of treatment. The extent of these differences among random comparisons within a rather brief period indicates that the effect of the unknown secondary factor or factors must be large. Since environmental conditions during irradiation of the pollen were substantially constant, and since they were necessarily variable during the ensuing period of pollen tube growth and early endosperm development, it seems probable that the environmental influence may be effective during some period subsequent to treatment. It is not impossible that conditions previous to treatment may be a factor, through their effect upon the final development of the pollen grain and consequently upon the absorption and internal filtration of the radiation. Correlation of the results with detailed weather records for the period under consideration showed no clear relation to specific changes of temperature or other weather conditions. The identification of the environmental conditions affecting the frequency of deficiencies must be left for further investigation.

Dosage Curves for Endosperm Deficiency

The dosage data for λ2652 and λ2967, presented in tables 4 and 5, include all treatments made at these doses during June and July 1938, the period in which the comparison of monochromatic radiations was made. Although an effort was made to seriate doses for comparison, it was not possible within the short period of high tolerance (June 21 to July 1) to obtain adequate numbers for the dosage comparison as well as the wave length comparison. The data on dosage obtained during this period were inadequate in number, especially for the larger doses, and were supplemented by the results of additional treatments made whenever the weather would permit during July. These data therefore are subject to error from daily fluctuation. The comparison of equal doses applied during the first period with those applied later, in the few cases in which significant numbers are available, indicates that the frequency of deficiency was lowered in the later supplementary treatments. Since the data for the higher doses are largely from these later pollinations, it is probable that the lower relative yield of large doses is due in part at least to this cause.

A special trial of the effect of dosage was therefore made, for λ2536, λ2967, and λ3022, during a period of four consecutive days (August 29 to September 1, 1938) with regular seriation of the doses used at each wave length. The results are shown in table 10.

The declining effectiveness of successive increments of dose is clearly evident in the results for λ2536. It is much less pronounced, if indeed it occurs at all, at λ2967 and λ3022. It is perhaps worthy of note that at both

TABLE 10

Relation of dosage to frequency of endosperm deficiency.
(Pollinations of August 29–September 1, 1938.)

DOSE (M)	NUMBER EARS	NUMBER DEFICIENT SEEDS	ENDOSPERM DEFICIENCIES								
			A		*Su*		*Pr*		TOTAL FREQUENCIES		
			E*	F*	E	F	E	F	NUMBERS	%±S.E.	α
					λ2536						
1.0	10	24	1	12	1	4	4	5	27/892	3.0±0.6	.0032
2.0	5	23	3	5	4	1	1	10	24/419	5.7±1.1	.0044
4.1	4	24	1	5	1	5	1	11	24/293	8.2±1.6	.0034
8.1	4	54	6	17	4	10	3	21	61/571	10.7±1.3	.0022
17.1	4	33	3	13	1	3	1	14	35/208	16.8±2.6	.0025
					λ2967						
4.0	5	13	0	5	1	2	1	5	14/543	2.6±0.7	.0003
7.9	8	77	8	34	8	11	4	19	84/786	10.7±1.1	.0009
15.7	4	46	3	23	2	9	5	13	55/305	18.0±2.2	.0008
33.3	8	121	7	55	4	23	6	42	137/420	32.6±2.3	.0010
					λ3022						
15.6	9	18	2	9	0	3	1	3	18/625	2.9±0.7	.0001
32.0	6	68	2	36	0	15	6	16	75/802	9.4±1.0	.0002
63.8	5	122	13	58	4	14	8	44	141/568	24.8±1.8	.0003
132.0	9	99	15	39	11	13	6	28	112/310	36.1±2.7	.0003

* E=entire; F=fractional

of these wave lengths there is again a suggestion of the disproportionately small effect of very small doses.

In general the frequency of deficiencies in this series of late-summer treatments is materially lower than that from equal doses in the early-summer treatments previously reported, another indication of the effect of environmental factors upon the frequency of induced deficiency.

Irradiation from Opposite Directions

If the diminishing return from successive equal increments of radiation is due to the varying depth dose at the position of the gametic nuclei, it should be possible to reduce this filtration loss by irradiating the pollen from different directions.

Let us first suppose that a sample of pollen is irradiated uniformly from above with a given dose of radiation and that each pollen grain is then tested for the occurrence of a specific effect, say deficiency for *A*. The pollen grains affected will tend to be those in which the nucleus was so placed as to be least shielded by overlying material, for the relative probability of effect in different nuclei will be dependent upon the relative intensity

of radiation to which they are exposed. Assume that ten percent of the pollen grains are affected. If now the sample is exposed to a second dose identical with the first, the increase in the frequency of affected pollen grains will tend to be less than the nine percent (ten percent of the remaining 90 percent) which would be expected if the pollen grains were uniformly susceptible to the treatment. The second treatment must produce its effects among those pollen grains which were unaffected by the first, and to the extent that these represent the grains with deeper-lying nuclei the second treatment will be less effective.

Consider next a pollen sample irradiated with the same total dose, but with the first treatment applied from above and the second from below. The pollen grains most favorably oriented for the first treatment are those least favorably oriented for the second, and *vice versa*. If differences in internal filtration are extreme, the yield of effects from the second treatment may be almost as large as that from the first, and the deficiency rate therefore may be nearly doubled.

Irradiation of pollen from opposite sides without disturbing orientation of individual grains was not feasible with the monochromator, but it could be performed conveniently with the mercury discharge tube which operates readily in any position. The discharge tube was mounted on a swinging arm which could be rotated so as to hold the tube in place either 21 cm above the pollen sample or an equal distance below. The pollen was exposed between quartz slides of equal thickness. Four pollen samples were irradiated simultaneously in each treatment, each on a separate slide, and one ear was pollinated from each sample. One treatment of 7.5 minutes' duration was made each day with radiation from above and a treatment of equal duration with radiation from below. The eight ears produced were taken together to represent the single dose. The double dose similarly represents four samples irradiated from above and four from below, all being irradiated for 15 minutes. The divided dose represents two treatments, in each of which the pollen was irradiated 7.5 minutes from above and 7.5 minutes from below. To obviate any day-to-day fluctuations in treating conditions or discharge tube output, the experiment was conducted so as to carry through the complete comparison in consecutive treatments, and the entire experiment was repeated on different days to accumulate sufficiently large numbers.

A single trial made in 1938 gave results in agreement with expectation as outlined above, but the numbers involved, particularly in the divided treatment, were inadequate. The control for this trial is the general control already described. Three additional trials were made in 1939, each with a separate control. The conditions were similar to those of the 1938 trials, except that in the 1938 trial the radiant surface of the source was

limited by covering a portion of the tube with adhesive tape, while in the 1939 trials the radiant surface was limited instead by a baffle of aluminum.

These four trials are reported separately in table 11.

TABLE 11

Enhanced frequency of endosperm deficiencies produced by a given dose when pollen is irradiated on opposite sides instead of on one side only.

EXPERIMENT NUMBER	TREATMENT	NUMBER EARS	NUMBER DEFICIENT SEEDS	ENDOSPERM DEFICIENCIES						TOTAL FREQUENCIES	
				A		*Su*		*Pr*			
				E*	F*	E	F	E	F	NUMBERS	%±S.E.
1	Half Dose (From One Side)	4	82	10	42	8	12	6	13	91/701	13.0±1.3
	Full Dose (From One Side)	6	68	8	42	4	17	2	9	82/374	21.9±2.1
	Full Dose (Half from Each Side)	4	36	2	9	1	13	2	12	39/137	28.5±3.9
2	Half Dose (From One Side)	8	191	15	69	14	22	15	83	218/840	26.0±1.5
	Full Dose (From One Side)	8	78	9	28	1	7	8	35	88/265	33.2±2.9
	Full Dose (Half from Each Side)	8	93	9	34	6	10	10	35	104/265	39.3±3.0
	Control	2	3	0	3	0	0	0	0	3/494	0.6±0.3
3	Half Dose (From One Side)	7	178	15	81	13	19	8	57	193/973	19.8±1.3
	Full Dose (From One Side)	8	116	13	53	13	11	6	35	131/472	27.8±2.1
	Full Dose (Half from Each Side)	8	61	10	24	4	6	8	18	70/187	37.4±3.5
	Control	3	3	0	3	0	0	0	0	3/284	1.1±0.6
4	Half Dose (From One Side)	8	86	8	38	7	10	6	26	95/648	14.7±1.4
	Full Dose (From One Side)	6	87	7	38	1	15	8	23	92/465	19.8±1.8
	Full Dose (Half from Each Side)	8	87	9	32	5	12	8	31	97/287	33.8±2.8
	Control	3	4	0	1	0	2	1	0	4/389	1.0±0.5

* E = entire; F = fractional

In each comparison the yield of deficiencies from the double dose applied from opposite directions is larger than that from the double dose applied from the same direction. The former treatment on the average produces almost twice as many deficiencies as the single dose, while the latter treatment produces only a small increase in the frequency. For the totalled data, the percentages of deficiencies for the single dose, the double dose, and the divided dose are 18.9, 24.9, and 35.4, respectively. The results thus indicate that the leveling of the dosage curve in this region is largely the result of selective effect of the radiation upon the pollen grains most favorably oriented.

Internal Filtration in Pollen

Maize pollen is approximately spherical with an average diameter of about 93μ. The surface of the pollen grain is smooth, and the thin wall is transparent in visible light. Since the reserve carbohydrate present is starch, the entire grains are somewhat translucent but not transparent in visible light. The pollen grain contains three nuclei, a large vegetative nucleus and two small, highly condensed sperm nuclei which are produced

by the division of the generative nucleus four or five days before maturity of the pollen grain. The structure of the pollen grain has been studied by WEATHERWAX (1919) and RHOADES (1934) who describe the sperms as thin crescent-shaped cells consisting almost entirely of nucleus but showing cytoplasm at their fine-pointed ends. The sperm nuclei are eccentrically located in the pollen grain, as may be seen readily in sectioned material or in specimens stained entire.

In order to obtain evidence on the approximate location of the sperm nuclei within the pollen grain, measurements were made upon mature pollen grains which had been stained and sectioned. We are indebted to MR. JAMES W. CAMERON for these measurements. Mature anthers, in which the pore had opened but the pollen grains had not been shed, were used in making the preparations. The material was sectioned at a thickness of 9–10 microns. The positions of the three nuclei were plotted in three dimensions for each of the 67 pollen grains examined, the position of each nucleus being recorded in one of five concentric shells. Although the two sperm nuclei sometimes differ somewhat in appearance, there is no way of discriminating between the one which will function in fertilization and the one which will function in endosperm fusion. The locations of the three nuclei were tabulated separately for each pollen grain, and the data thus constitute a sample of 67 vegetative nuclei and 134 sperm nuclei. The frequency distribution of their locations in the five concentric shells is shown in table 12. Both the vegetative nucleus and the sperm nuclei are eccentric in location, but in general the sperm nuclei are closer to the periphery of the pollen grain. Only about six percent of the sperm nuclei are located in the two innermost shells, while more than 30 percent of the vegetative nuclei occupy this position. In general the two sperm nuclei in the same pollen grain were about equally eccentric, the two nuclei being in the same or adjacent shells in more than half of the pollen grains examined.

TABLE 12

Location of nuclei within the pollen grain.

CONCENTRIC SHELL	VEGETATIVE NUCLEUS		SPERM NUCLEI	
	NUMBER	%	NUMBER	%
1 (Innermost)	1	1.5	2	1.5
2	20	29.9	6	4.5
3	27	40.3	35	26.1
4	18	26.9	68	50.7
5 (Outermost)	1	1.5	23	17.2
Total	67	100	134	100

Assuming random orientation of pollen grains lying in a single layer, the vertical depth distribution of sperm nuclei beneath the upper hemispherical surface of the pollen grains may be calculated. The distribution represented in figure 1 indicates the relative numbers of nuclei per unit depth in a large sample of pollen, where each successive layer of unit depth is bounded by *hemispherical* shells of constant radius, with all points on any one shell lying at equal distances vertically from the upper surface of the pollen grain.

Ultraviolet transmission data on the walls and contents of the pollen grains have been published by UBER (1939). Transmission measurements were made with a quartz microscope for various wave lengths from 2378 to 3655 Å. Fragments of individual walls were obtained by squeezing the contents out of the pollen grains. Transmission data for the pollen contents were obtained from layers eight microns thick pressed between quartz slides. The transmission data for the wave lengths used in the present experiment are shown in table 13.

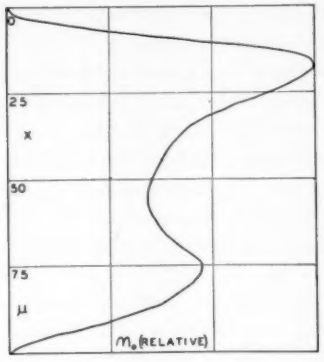
TABLE 13

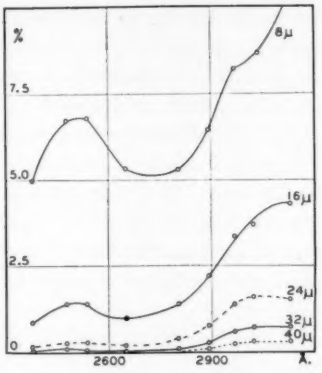
Ultraviolet transmission by pollen grain wall and contents.

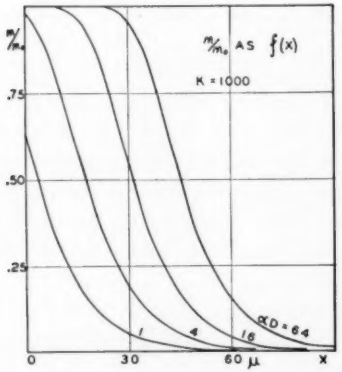
λ	WALL %	CONTENTS (PER 8μ LAYER) %	$k\left(=\frac{\ln(I_0/I)}{0.0008}\right)$
3131	25	41.5	1100
3022	20	43	1055
2967	20	41	1115
2894	19	34	1350
2804	20	26.5	1660
2752	23	23	1835
2652	28	19	2075
2536	33	20.5	1980
2483	32	21	1950
2378	28	18	2145

Transmission through the pollen wall is at a minimum of about 20 percent at 2800–3000 Å and reaches a maximum of about 33 percent at 2536 Å. When equal doses of λ2536 and λ2804 are applied, therefore, the dose which reaches a point immediately under the pollen wall is almost twice as great for the shorter wave length as for the longer. Transmission through an eight μ layer of pollen contents is only 19 percent at λ2378 and at λ2652, but amounts to about 42 percent at λ3022. Corresponding differences in intensity would occur at a point eight microns below the wall and would be increased exponentially at greater depths.

The relative proportion of incident energy which penetrates to various depths within the pollen grain, neglecting corrections for reflection and refraction, is shown in figure 2. Thus doses equal in incident energy are far from equal in the energy which reaches the sperm nuclei. With equal incident energy at λ2967 and λ2652, the dose applied at a point only 16 microns







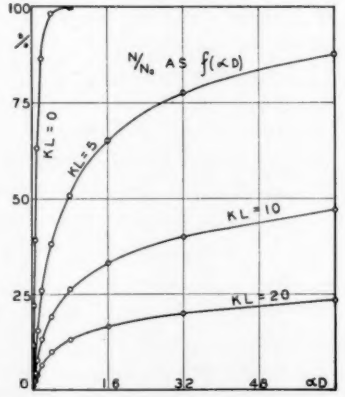


FIGURE 1 (upper left).—Relative distribution of nuclei (abscissa) in a pollen sample at depths (x) of equal energy penetration for radiation incident vertically from above.

FIGURE 2 (upper right).—The percent of incident energy (ordinate) which penetrates to the indicated depths within a pollen grain as a function of the wave length (abscissa). Absorption by the pollen grain wall has been taken into account.

FIGURE 3 (lower left).—The fraction of nuclei affected (n/n_0) by radiation at various depths within a pollen grain for several values of αD and for $k = 1000$.

FIGURE 4 (lower right).—The percentage of nuclei affected (N/N_0) in a large sample of pollen as a function of αD for several values of kL. where L is the diameter of a pollen grain.

beneath the wall is three times as large for the longer wave length as for the shorter. At a depth of 32 microns it is about fifteen times as large.

Relation of Internal Filtration to the Dosage Curve

If the nuclei in a pollen sample (or more strictly the absorbing entities within them which are responsible for the occurrence of deficiencies) were so disposed as to be equally shielded from the radiation, internal filtration would have no effect upon the form of the dosage curve. Each unit of radiation incident at the surface of the pollen grain would have the effect of only that fraction of a unit which penetrates to the site of nuclear absorption. If this fraction were constant, the dosage curve stated in terms of incident energy would be correct in form; it would be in error only as to the absolute value of the dosage unit.

Variation in the thickness of the shielding layer, however, may result in a distortion of the dosage curve. Due to this variation, the nuclei in a sample of pollen grains exposed to a uniform beam of radiation may constitute in fact a population of individuals receiving widely varying doses. The incident dose on the pollen is uniform for all grains, but it is reduced in amount within each individual grain depending upon the depth of the gametic nucleus below the exposed surface (figure 1, table 12) and on the ultraviolet transmission characteristics of the pollen grain wall and contents (fig. 2, table 13).

The effect of this variation in shielding upon the dosage curves for incident radiation is to reduce the relative effectiveness of the later dose increments. With equal shielding, the probability of affecting a nucleus not previously altered by the radiation would be the same for successive dose increments. With unequal shielding, this probability declines with each increment of dose applied, since each dose increment tends to affect most the least shielded pollen grains and to leave unaffected a residual population with fewer nuclei in the less shielded positions. The extent of this tendency will vary with different wave lengths, depending upon (1) the rate at which the energy is lost in penetrating the over-lying material and (2) the genetic effectiveness of the energy which reaches the nucleus.

The amount of modification expected in the dosage curve for deficiencies at each wave length may be estimated in terms of two variables, k and α. The first variable, k, is defined as an absorption coefficient for the pollen contents by the equation, $I = I_0 e^{-kx}$, where x is the thickness of the absorbing layer, and I and I_0 are the transmitted and incident intensities, respectively. The value of k is known, to a sufficient approximation, from data on absorption of the pollen contents and is tabulated in table 13. Since much of the radiation is scattered by the starch granules in pollen,

k is not strictly an absorption coefficient, but includes also the radiation loss by scattering.

The second variable, α, is a proportionality constant in the following fundamental equation (cf. CLAUS 1933): $dn/dt = \alpha(n_0 - n)\ I_0\ e^{-kx}$. This equation assumes that the time rate of production of deficiencies (dn/dt) depends only on the intensity of the radiation reaching the germinal material (I_0e^{-kx}) and on the number of unaffected centers in the population ($n_0 - n$) at the time (t). Since dn/dt is independent of the total dose ($D = I_0t$), this case is usually referred to as one requiring only a single absorption act to bring about a deficiency.

Intrinsically the proportionality constant, α, depends on two factors: (1) the probability of absorption of the energy incident on a potentially effective center or region, and (2) the probability of production of a detectable genetic alteration by the energy which is absorbed. The second factor is analogous to the quantum yield of a photochemical reaction and concerns us at this point only as regards its possible variation with wave length. In the absence of available information as to wave length dependence, it has been customary in studies of this nature to assume constancy over the ultraviolet region investigated. This assumption is probably not entirely justified but will serve as a first approximation.

The fraction of the incident energy which is absorbed by a substance occupying a small volume is equal to $1 - e^{-\xi cd}$, where ξ, c, and d represent its molecular extinction coefficient, its concentration, and its depth, respectively. In case ξ c d is small compared to unity, which would presumably be true for minute absorbing structures, this fraction becomes equal to ξ c d, and hence the energy absorbed would be proportional to the coefficient ξ. In other words, to a first approximation, α should be proportional to the absorption coefficient of the substance responsible for the production of the deficiencies.

Our problem, then, is to determine a relationship between the several variables so that the value of α may be obtained from the curves relating deficiencies to the dose incident upon the pollen at each wave length.

For a population which is uniformly shielded by a substance having an extinction coefficient, k, such as the population (n_0) of a layer dx thick at a depth x, the number of deficiencies (n) produced in the layer is given by $n = n_0(1 - e^{-\alpha De^{-kx}})$. Values of n/n_0 as a function of x for various values of αD are given for the case where $k = 1000$ in figure 3. It will be noted that when αD is large practically all of the nuclei near the surface are affected. Since additional increments of dose cannot increase n/n_0 in these uppermost layers, the dosage curve becomes proportionately less steep at the larger doses.

Inasmuch as the experimental data on deficiencies represent the effects on the population as a whole (N_0), we must sum up the effects expected from the different "layers" of the pollen grain. The relative distribution of nuclei among the various layers is shown in figure 1, where x represents the vertical depth of a layer below the upper wall of the pollen. If we multiply the actual number of nuclei at any depth x by the above value of n/n_0 for the same depth, the product is the number of nuclei affected at that depth. By plotting the values thus obtained against the depth x and integrating graphically from O to the diameter L, the total number of deficiencies, N, is found. A series of values for N/N_0 as a function of αD may then be calculated. The resulting dosage curves for several values of kL, shown in figure 4, indicate how marked the levelling of the dosage curve may be, for a kL value of 10 corresponds to λ3022 and a kL value of 20 to λ2652.

The experimental values of N/N_0 for endosperm deficiencies as a function of dose for three different wave lengths have been given in table 10. Taking any particular value of N/N_0 for which D is known, it is possible to determine α from the appropriate curve of the type shown in figure 4. This has been done, using as a basis the dose just inside the pollen wall, and the values of α are given in table 10.

According to this analysis, the values of α for different doses of the same wave length should be equal, except for sampling fluctuations. The calculated values show considerable variation, and in the case of λ3022 and λ2967 they tend to increase somewhat with increasing dosage. The extent to which these variations may be accounted for by sampling errors may conveniently be tested by constructing a predicted dosage curve at each wave length, against which the experimental values may be plotted with their standard errors. Predicted dosage curves for the wave lengths appearing in table 10 are presented in figure 5; these curves have been constructed on the basis of the α values yielding the best fit.

At all wave lengths except λ3022 the observed deficiency rates are in reasonably good agreement with prediction. At λ3022 the data clearly depart from expectation, the deficiency frequency being distinctly too low at the smaller doses and too high at the larger doses. Such behavior suggests a multiple-hit curve for the dosage relation.

In the light of this indication, and in view of earlier remarks on the shape of the dosage curve for endosperm deficiencies, it seemed profitable to calculate values of α on the assumption that the cumulative effect of two quanta would be required to give an effect. In this case the equation for the number affected (n_2) in a thin layer becomes

$$n_2 = n_0[1 - e^{-\alpha De^{-kx}}(1 + \alpha De^{-kx})].$$

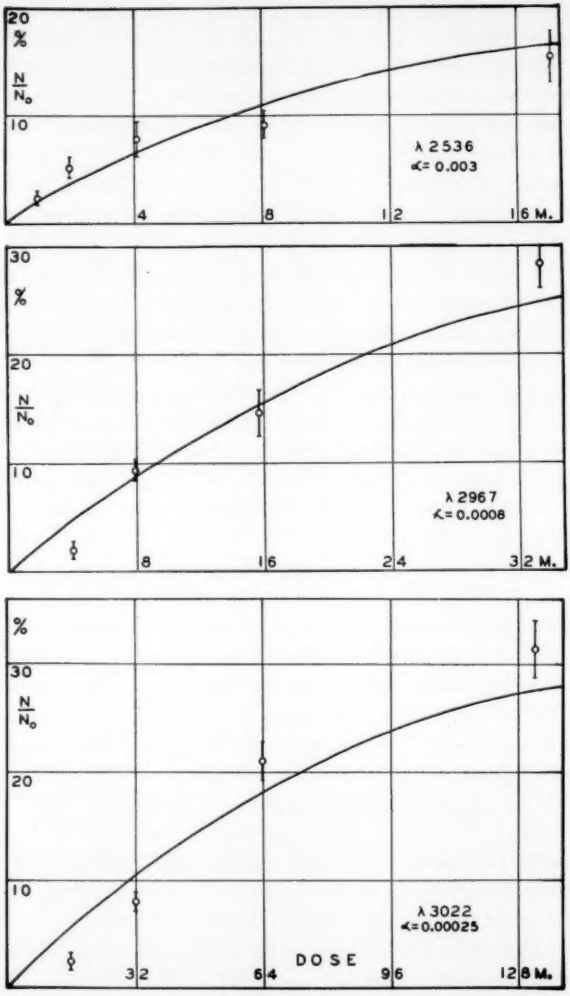


FIGURE 5.—The experimental values of the frequency of deficiencies (N/N_0) as a function of the incident dose in comparison with the theoretical curves of the expected variation of N/N_0 with D. The curves are based on the arbitrary values of α which are indicated on each of the curves.

Integration over the total depth of the pollen grain results in slightly S-shaped curves for N/N_0 as a function of αD, from which values of α may be computed as previously described. When this was done, the constancy of α was found to be somewhat better at λ2967 and λ3022 for the 2-quanta

case than for the single quantum treatment, while at λ2536 the reverse was true.

While the improvement noted in the fitting of these curves is not necessarily significant, the possibility of a multiple-hit type of relationship between deficiency rate and dosage certainly merits further investigation.

Comparative Effectiveness of Different Wave Lengths

Comparisons of treatment at different wave lengths were made on a rather large scale during June and July, 1938. The most extensive data for this purpose were obtained at a dose of 2 M, but fairly extensive comparisons were made also at 8 M. The treatments at ½ M, which have been summarized in an earlier table, resulted in such small increases over the control frequency that the differences between wave lengths have little statistical significance.

The totalled data for wave length comparisons at 2 M and 8 M are shown in table 14.

TABLE 14

Relation of wave length to frequency of endosperm deficiency.

WAVE LENGTH (Å)	DOSE (M)	NUMBER EARS	FREQUENCIES OF EMBRYO ABORTION		NUMBER DEFICIENT SEEDS	ENDOSPERM DEFICIENCIES						TOTAL FREQUENCIES	
						A		*Su*		*Pr*			
			NUMBERS	%±S.E.		E*	F*	F	E	E	F	NUMBERS	%±S.E.
3022	1.98	14	3/820	0.4±0.2	15	0	6	2	1	2	4	15/1696	0.9±0.2
2967	1.99	11	5/1006	0.5±0.2	67	3	33	4	12	3	15	70/1498	4.7±0.5
2925	2.00	3	0/270	0	43	1	20	2	8	1	14	46/510	9.0±1.3
2894	1.91	8	7/946	0.7±0.3	55	1	31	2	6	5	18	63/1010	6.2±0.8
2804	1.97	7	8/1016	0.8±0.3	86	6	44	7	17	5	15	94/1016	9.3±0.9
2752	2.03	3	0/91	0	32	2	10	3	13	5	6	39/399	9.8±1.5
2699	1.96	2	0/0		19	5	4	1	6	0	5	21/268	7.8±1.6
2652	1.99	9	23/1107	2.1±0.4	102	9	47	8	25	6	28	123/1063	11.6±1.0
2536	1.96	7	17/736	2.3±0.6	108	11	50	8	19	5	31	124/802	15.5±1.3
2483	1.96	9	13/762	1.7±0.5	89	12	44	9	17	5	11	98/987	9.9±1.0
2378	2.04	2	0/150	0	15	0	8	1	1	2	4	16/231	6.9±1.7
2967	7.69	6	16/684	2.3±0.6	74	6	34	3	12	7	16	78/684	11.4±1.2
2894	7.82	6	34/582	5.8±1.0	109	11	60	5	16	9	24	125/675	18.5±1.5
2804	7.97	6	48/648	7.4±1.0	180	16	76	11	45	7	47	202/851	23.7±1.5
2699	8.05	5	45/450	10.0±1.4	81	6	40	5	12	6	25	94/450	20.9±1.9
2652	7.85	9	70/927	7.6±0.9	124	12	62	7	29	4	27	141/927	15.2±1.2
2536	7.82	6	44/383	11.5±1.6	81	4	28	9	19	8	21	89/383	23.2±2.2
2483	7.94	7	20/184	10.9±2.3	67	4	36	5	18	5	11	79/346	22.9±2.3

* E = entire; F = fractional

These data involve the same error from daily fluctuation which has been discussed in connection with the totalled dosage data. They will serve, however, for the consideration of questions involving embryo abortion and the relative frequency of fractional and entire deficiencies.

Embryo abortion is distinctly more frequent at the shorter wave lengths. The increased frequency is appreciable at the 2 M dose at λ2652–λ2483 but

not at longer wave lengths. With a dose of 8 M, appreciable embryo abortion occurs at all wave lengths, but it is more frequent at the shorter wave lengths than at the longer.

The relative frequency of fractionals as a function of wave length is of interest in connection with the problem of their origin. Fractionals are much more frequent with ultraviolet treatment than with X-ray treatment, and in the case of ultraviolet fractionals the sizes of the deficient and non-deficient sectors are usually approximately equal. The ultraviolet

TABLE 15

Relation of Frequency of Deficient Seeds to Wavelength

(*Dose 2 M*)

λ	FREQUENCIES ON THE DATE INDICATED			MEAN	(N/N_0) CALC.	α
	6/24	6/25	6/27			
3022	4/462	6/621	5/613	0.9±0.2	1.0	0.0001
2967	9/266	35/459	17/600	4.6±0.6	5.1	0.0013
2894	5/89	8/95	37/512	7.2±1.0	6.8	0.0043
2804	9/111	24/180	15/249	8.9±1.2	9.4	0.0082
2752	14/265	5/43	13/91	8.0±1.4	10.2	0.0082
2652	16/183	14/129	43/266	12.6±1.4	11.8	0.0166
2536	14/140	33/205	41/289	13.9±1.4	13.0	0.0169
2483	10/140	25/168	37/353	10.9±1.2	11.7	0.0115
2378		5/81	10/150	6.5±1.6	7.3	0.0061
Control		15/2075	1/585	0.6±0.2		

fractionals may represent the result of breakage of the half-chromosome, in contrast to the entire deficiencies resulting from breakage of the undivided chromosome or of the two half chromosomes. If fractionals result from absorption of the radiation in different parts of the chromosome and if these parts differ in absorption characteristics, we might expect a different ratio of entire and fractional deficiencies at different wave lengths. The data however give no indication of any such difference.

For calculation of relative genetic effectiveness of the wave lengths it is desirable to limit the data to those which are the result of experiments seriated for the control of daily variation. In the case of the treatments at a dose of 2 M, fairly extensive data are available from seriated treatments, and they are presented in table 15.

The relative effectiveness of the wave lengths, corrected for loss due to internal filtration, may be compared by calculating the value of α for each wave length in the manner which has been described. These values are also listed in table 15. The calculations have been made on the basis of

the dose expressed in terms of total energy rather than total number of quanta.

These values of α are plotted against the absorption spectrum of thymonucleic acid (CASPERSSON 1936) in figure 6. Considering the various approximations involved, the agreement is surprisingly good and lends further support to the frequently advanced hypothesis that nucleic acid is intimately associated with the functioning of the germinal material.

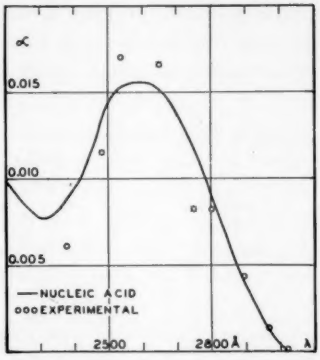


FIGURE 6.—Experimental values of α (ordinate) are plotted against the wave length (abscissa) for comparison with the absorption spectrum of thymonucleic acid (CASPERSSON 1936) the latter being shown as a full curve.

The significance of the deviations with relation to sampling error may be determined by comparison of the observed deficiency rates with values given in table 15 under "N/N_0 calculated." These are calculated on the assumption that the genetic effectiveness of the energy that reaches the gametic nucleus at any wave length is proportional to the absorption of that wave length by nucleic acid. The experimental values do not deviate significantly from the calculated values.

The Genetic Effectiveness of λ3131

The earlier comparison (STADLER and UBER 1938) showed no appreciable effect of λ3131 on endosperm deficiency rate. At a dose of 600 M there was a distinct increase in the frequency of deficiencies (see table 1), but since impurities of shorter wave length radiation were present in sufficient strength to constitute a considerable dose in this very long exposure, the genetic effects could not be ascribed definitely to λ3131. The impurity normally present, totalling all wave lengths shorter than λ3131, amounted to less than one percent. It was not feasible to use a double monochromator to increase the purity of the radiation, since the dose required for genetic

effectiveness is so large that the energy available would be inadequate within the time limits.

It was found, however, that the impurity could be very materially reduced without serious reduction in the intensity of the desired radiation by the use of a Corning #014 glass filter (see table 2). By the use of this filter, radiation impurities of wave lengths shorter than 2900 Å are reduced to an entirely negligible value, and that due to λ3022 and λ2967 is seen to be very small. In order to ascertain the deficiency rate which could result from such slight percentages of impurity, special treatments were given at λ3022 and λ2967 for doses of approximately $\frac{1}{2}$ M and $\frac{1}{4}$ M, respectively. Even these low doses are greatly in excess of the impurity from these lines

TABLE 16

Data relative to the genetic effectiveness of λ3131.

TREATMENT		NUMBER EARS	NUMBER DEFICIENT SEEDS	ENDOSPERM DEFICIENCIES						TOTAL FREQUENCIES	
				A		*Su*		*Pr*			
λ Å	DOSE M			E*	F*	E	F	E	F	NUMBERS	%±S.E.
3131†	505.2	16	27	1	12	0	8	2	4	27/1641	1.78±0.23
3022	0.46	17	15	0	10	0	1	2	2	15/2092	0.72±0.12
2967	0.25	18	9	1	3	0	1	0	4	9/1618	0.58±0.13
Control		12	5	0	2	0	0	0	3	5/1500	0.33±0.08

* E=entire; F=fractional

† Filtered (See text and Table 2.)

found to be present in a dose of 512,000 ergs/mm² at 3131 Å. The data on the control frequency of endosperm deficiencies were obtained from simultaneous pollinations, the pollen being exposed in trays near the monochromator for the same period of time as the λ3131 samples. The exposure time varied from 20 to 30 minutes, depending on the intensity of the radiation. The data are presented in table 16.

The deficiency rates for the low doses at λ3022 and λ2967, from which the control frequency has not been subtracted, will be seen to be too low to account for the effect at λ3131. Apparently this wave length has a real though very slight effect on the frequency of endosperm deficiency.

SUMMARY

The results of comparisons of the effectiveness of different wave lengths differ rather widely in trials made at different levels of dosage. The problem of comparing wave length effectiveness is therefore chiefly that of analyzing the dosage relation.

In general the dosage curve for ultraviolet radiation tends to level at the larger doses; the return from large doses is proportionately less than

from small. This tendency is more pronounced at λ2536 and λ2652 than at λ2967 and λ3022.

The disproportionately small return from an increment of dose may be avoided if the increment is applied from the opposite side of the pollen grain, indicating that variations in the susceptibility of the pollen grains treated are related to their orientation.

The gametic nuclei within the pollen grains are eccentrically located, so that in a layer of pollen grains oriented at random there are large differences in the depth of the nuclei below the irradiated surface.

The absorption of ultraviolet radiation in the pollen wall and contents is great enough to cause large differences in the intensity of the radiation penetrating to the site of the nucleus in different pollen grains. Transmission losses are greatest for the shorter wave lengths, at which the levelling of the dosage curve is most pronounced.

Using observed values for the position of the nuclei within the pollen grain and for the transmission losses in pollen wall and contents, and assuming that the effectiveness of each wave length per unit of energy is constant, it is possible to determine the form of the dosage curve expected at each wave length. The amount of levelling in the calculated dosage curves was in fairly good agreement with that observed.

The value of a constant representing the effectiveness per unit of energy reaching the nucleus was determined for each of nine wave lengths in the range λ2378–λ3022, from data representing the effect of doses of 2000 ergs/mm² of energy incident at the surface of the pollen grain. These values agreed within the limits of sampling error with the relative absorption coefficients of nucleic acid for the same wave lengths.

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A COMPARATIVE STUDY OF CERTAIN HETEROCHROMATIC REGIONS IN THE MITOTIC AND SALIVARY GLAND CHROMOSOMES OF DROSOPHILA MELANOGASTER

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INTRODUCTION

A MARKED secondary constriction exists in a mitotic chromosome of *Drosophila melanogaster* (BRIDGES 1927; HEITZ 1933). It was found by KAUFMANN (1933) to be in the left arm of the second chromosome. It is not associated with a nucleolus-forming region (KAUFMANN 1934). In prophase stages, the parts of the chromosome adjoining the constriction may be widely separated, sometimes by almost the width of the nucleus. Its presence and relative position are constant features of the prophase chromosome; it constitutes a definite landmark. No evidence of the constriction is discernible in the salivary gland chromosomes, however. The present study is concerned with the location of this region in the salivary gland chromosomes and with the comparison of the relative lengths of regions adjacent to the constriction in both the mitotic and salivary gland chromosomes. The results were partially published in abstract (HINTON 1940) and included in the report of DEMEREC, KAUFMANN, SUTTON, and HINTON (1940).

MATERIALS AND METHODS

In order to determine the location of the secondary constriction in the salivary gland chromosome, rearrangements involving various lengths of the left limb of the second chromosome (IIL) were studied in both mitotic and salivary gland preparations. Neuroblast cells of the larval ganglion were used for mitotic figures. The acetocarmine method was used throughout.

Although, as has been stated, the constriction does not appear in salivary gland chromosomes, it was possible by cytological observation to determine whether breaks which lie in the IIL arm are proximal or distal to the constriction as seen in the mitotic chromosomes. In this manner the region of the salivary gland chromosome which encompassed that portion of the mitotic chromosome containing the constriction could be located. Once these boundaries were established, the region between the constriction and any given break on IIL could be measured on the mitotic chromosome

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and compared with the relative lengths of the corresponding region on the salivary chromosome.

Stock 264-59 (Cold Spring Harbor stock list, Drosophila Information Service No. 12) is a reciprocal translocation between IIL (the break in 40F) and X (at 3C-8) (BRIDGES's 1935 map). In ganglion cells containing this translocation it has been seen that the constriction remains with the portion of the second chromosome having the centromere, and that the tip of X (3C-8 to tip) is attached distal to it. This piece, from the constriction to the tip of X was measured. Other stocks were available in which that portion of the X from the 3C region to the tip was reciprocally translocated with various parts of IIL. In order to determine the length of any section of IIL involved in such a translocation, measurements were made of the distance between the constriction and the tip, and from this was subtracted the measurement obtained from stock 264-59. This method was used to determine the length of the section from 34C7-D, 40C-D, 40E-F, and 40F to the base of IIL. The section 36F to 40A was measured by its absence from the chromosome, it having been inserted into X.

The data were compiled with the aid of a camera lucida (×4500). All measurements were made on the drawings and converted into percentages of the length of the X chromosome in the figure drawn in order to establish a constant. Series of measurements were made for each aberration, and from them an average was obtained. The final data are in microns and represent the length of the chromosomes when the X measured 3.5 microns (the average for 39 X chromosomes). As an example, 3C8 to the tip of the X chromosome measures (from the camera lucida drawings) .14X or .14 times 3.5 microns or 0.5 micron.

In order to locate the secondary constriction in the salivary gland chromosomes by the method outlined above, the following stocks of Drosophila were used: 264-50 (part of X, 3C7 and 9-10 to 20C1-2, inserted into IIL at 22A-B); 264-80 (IIL, 36F to 40C inclusive, inserted into X at 3C5-6 and 7); 264-74 (IIL from 40C-D to tip translocated to 20D-E of X and replaced by 3C9-10 to 11 to tip of X—the piece, 20D-E to 3C of X, inserted in IIIR at 92E); 258-39 (reciprocal translocation, X at 3C2-3 and 5-6 and IIL at 40E-F); and 264-59 (a reciprocal translocation, X at 3C8, and IIL at 40F). Stocks which were used in addition to the above to measure the various regions of the chromosome include: 264-53 (a reciprocal translocation, X at 3C5-6-7, and IIL at 34C7-D); 264-23 (a reciprocal translocation, X at 3C8 and 9-10, and IIR at 41A); and 260-2 (a tip deficiency for the X chromosome—1B1-2 and 3-4 to tip) (DEMEREC and HOOVER 1936).

The stocks of Drosophila carrying these aberrations were furnished by DR. M. DEMEREC.

RESULTS

When sections near the tip of the second chromosome were removed by translocation, the constriction was not involved. When section 36F to 40C, inclusive, was removed, the constriction was not affected. Since 40C is separated from the centromere of IIL only by subdivisions 40D-F of BRIDGES'S map, this evidence indicated that the constriction must lie somewhere in the proximal part of the 40 region. Further study showed that when the section of IIL from 40C-D to the tip was transferred by translocation, the constriction still remained with the centromere of IIL. Sections from 40E-F to the tip and finally 40F to the tip were removed,and still the constriction remained with the centromere-bearing portion (fig 1).

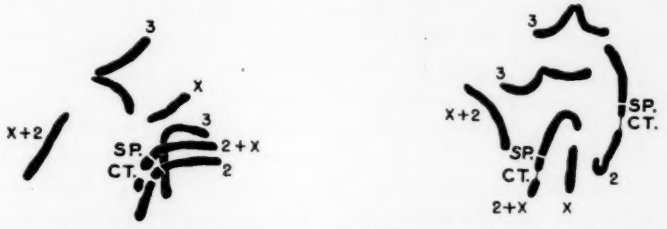

FIGURE 1.—Camera lucida drawings showing a reciprocal translocation in which the X chromosome (3C8 to the tip) has replaced the left arm of the second chromosome (40F to the tip). The constriction remains with the centromere of the second chromosome. Stock 264-59. SP = Spindle-attachment (centromere); CT = secondary constriction.

Thirty-two mitotic plates were interpreted and drawn from slides on translocation 264-59; five to fifteen mitotic figures were obtained for each of the other aberrations.

The bands in 40F are the most proximal bands of IIL. At least all but one band on the map of IIL were removed without affecting the constriction and the region between the constriction and the centromere. It is possible that part of the 40F region remained, since it is very diffuse in appearance and not readily analyzed. Nevertheless, the evidence shows that the region of the mitotic chromosome between the constriction and the centromere (about one-fifth of the left arm) is represented on the salivary gland chromosome by, at most, one band.

When the measurements for the other sections of the left arm were obtained from the mitotic chromosomes (table 1), it was found that the section extending from 40D to 40F of the salivary gland chromosome represented a relatively large section in the mitotic chromosome. The section between 40D and 40F represents less than one-twentieth of the length of IIL in the salivary gland chromosome. It is represented by about one-fifth of the left arm of the mitotic chromosome.

TABLE 1

Measurements of normal and rearranged mitotic chromosomes.

CHROMOSOME OR SECTION MEASURED	LENGTH IN MICRONS	MEAN LENGTH IN TERMS OF X CHROMOSOME	STOCK USED IN THE MEASUREMENT
IIL (constriction contracted)	2.9	.84X	All stocks studied
Constriction	.5	.14X	All
From constriction to centromere of 2	.5	.15X	All
IIR	2.8	.81X	All
III	5.2	1.49X	264-74
Tip of X to 3C5-6 plus 34C-D to centromere of II	1.9	.54X	264-53
From cent. of X to 3C7 plus 34C-D to tip of IIL	4.6	1.31X	264-53
X with 36F to 40D inserted at 3C5-6 to 7	3.7	1.08X	264-80
IIL with 36F to 40D missing	2.7	.76X	264-80
Cent. of IIL to 40C-D plus X 3C9-10 to tip	1.6	.45X	264-74
Cent. of X to 20D-E plus 40C-D to tip IIL	2.3	.66X	264-74
III with 20D-E to 3C11 of X inserted	7.8	2.22X	264-74
Cent. of II to 40E-F plus 3C2-3 to tip X	1.3	.37X	258-39
Cent. of X to 3C5-6 plus 40E-F to tip of IIL	5.1	1.45X	258-39
Cent. of II to 40F plus 3C8 to tip of X	1.0	.29X	264-59
Cent. of X to 3C9-10 plus 40F to tip of IIL	5.1	1.46X	264-59
Cent. of II to 41A plus 3C8 to tip of X	.9	.25X	264-23
Cent. of X to 3C9-10 plus 41A to tip of IIR	5.4	1.53X	264-23
X minus tip to 1B1-2	3.2	.90X	260-2
X	3.5		
Normal X which appears shorter than other X in same cell	3.2	.92X	wild stock
Lengths derived from the above measurements			
Tip of X to 3C8	.5	.14X	
3C11 to 20D	2.5	.72X	
20E to cent. of X	.5	.13X	
Tip of IIL to 34C	1.5	.42X	
36F to 40D	.3	.08X	
34C to 40D	.3	.09X	
40D to 40E	.4	.10X	
40F region	.2	.06X	
41A to cent. of IIR	.4	.12X	

The region, 40D to the base of IIL, is probably the heterochromatic part of the salivary gland chromosome due to its diffuse appearance and to its pairing properties with similar regions of other chromosomes. Al-

though it comprises less than one-twentieth of the salivary IIL, it occupies about two-fifths of the mitotic IIL. The heterochromatic region of the mitotic IIL is, thus, composed of at least two regions of heterochromatin, one of which is represented by a relatively small amount in the salivary gland chromosome as compared with the euchromatic regions, and another which is not visibly represented by more than one band.

The euchromatic sections of the mitotic IIL, naturally, show the reverse of the reduction found in the two heterochromatic regions, when represented on the salivary gland chromosome. These euchromatic sections occupy relatively small areas on the mitotic chromosome. Thus the section 34C-D to 40C-D (about three-tenths of the salivary IIL) is represented by only about one-tenth of the mitotic IIL.

In a similar manner all but the most proximal section of IIR was removed in a translocation (264-23), and the amount of the mitotic chromosome remaining attached to the centromere was measured. The piece which remained was equal to three-twentieths of the arm. This finding was independent of SCHULTZ's discovery of a similar region in IIR, the report of which appeared recently (MORGAN, SCHULTZ, and CURRY 1940). Likewise, the section from 20D-E to the tip of the X chromosome was removed (264-74), and a piece about one-fifth of the mitotic X chromosome remained with the centromere. How much of this, however, corresponds to divisions E and F cannot be said. This suggests regions similar to those found by MULLER and GERSHENSON (1935).

These measurements indicate, therefore, that the second chromosome in mitotic figures has a region extending across the centromere comprising over three-twentieths the total length of the chromosome, which is represented on the salivary gland chromosome by, at most, one band on each side of the centromere. The left arm of the second chromosome is shown to contain another region of heterochromatin which forms the remainder of the heterochromatin of the salivary chromosome.

Figure 2 shows the proximal regions of the second chromosome in the salivary gland chromosomes plotted against the same regions of the mitotic chromosomes. In order to make this comparison, the mitotic X chromosome was given the value of 14.75 inches (the length of the salivary X chromosome on BRIDGES'S 1935 map) and the other chromosome sections drawn to scale.

Since the heterochromatin has been shown to comprise a larger portion of the mitotic than of the salivary gland chromosomes, it was thought that terminal heterochromatin might be demonstrated if the normal X chromosome were compared to a terminally deficient X. For this purpose, a terminal deficiency stock, 260-2, was utilized (DEMEREC and HOOVER 1936). The average length of the chromosomes which appeared shortest of the two in

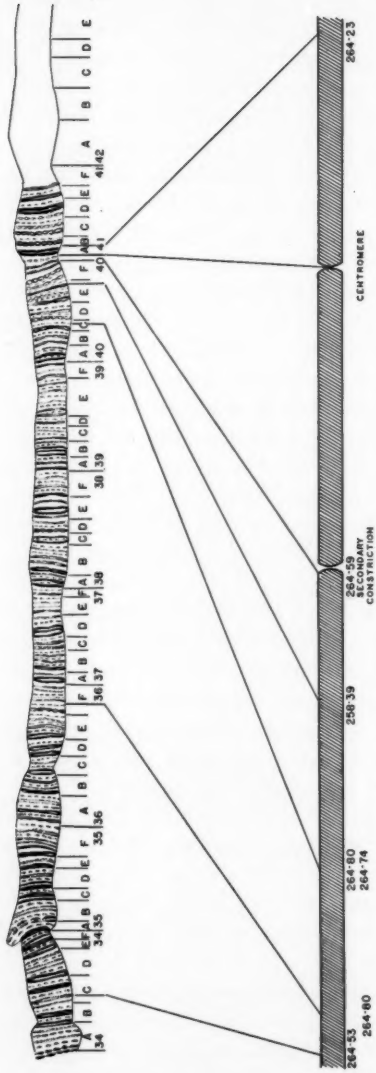

FIGURE 2.—Comparison of the second chromosome in salivary gland and mitotic chromosomes. Stocks used in the determinations and the actual measurements are given in table 1.

each mitotic figure of stock 260-2 was 3.2 microns. The average length of the longer X chromosomes was 3.5 microns. This difference is probably not significant when compared with a similar measurement from a stock with both X chromosomes normal.

DISCUSSION

The present findings show that a large part of the heterochromatin adjacent to the centromere on each arm of the second chromosome in *Drosophila melanogaster* is represented by no more than one band in each arm of the salivary gland chromosome. The remainder of heterochromatin accounts for the other bands in the heterochromatic region of the salivary chromosome.

These two regions of heterochromatin differ in their staining reaction in mitotic prophase chromosomes. It was reported by HEITZ (1933), by KAUFMANN (1934), and by PROKOFYEVA (1934) that sections adjacent to the centromere in mitotic prophase figures are heteropycnotic. The present study indicates that the region observed by these workers in the left arm of the second chromosome as being heteropycnotic is represented in the salivary chromosome by, at most, one band. It also indicates that the bulk of the heterochromatin in the left arm of the second chromosome of the salivary gland nucleus is formed from chromatin which stains in the mitotic prophase nucleus like the euchromatin. Since the heteropycnotic region appears to act as a unit, it is possible that the more distal heterochromatin is composed of similar, but smaller, units, each forming one band. This idea agrees with the prior findings of MULLER et al. (1935, 1937) for the heterochromatic region of the X.

Evidence has been reported (KAUFMANN and DEMEREC 1937; BAUER, DEMEREC, and KAUFMANN 1938; BAUER 1939) indicating that the number of breaks caused by irradiation in the heterochromatin is proportional to the length of heteropycnotic material observed in the mitotic chromosomes. It was concluded from this that euchromatin and heterochromatin are broken by X-rays with an equal frequency. MULLER et al. (1935, 1937) obtained evidence against this from the X chromosome. Moreover, if, as indicated here, the heterochromatin of the salivary IIL includes the heteropycnotic region of the mitotic IIL plus an equally long region which takes a stain similar to euchromatin, then the frequency of breaks detected in the heterochromatin of the salivary gland chromosome is proportional to only half the length of the heterochromatin of the mitotic chromosome. Therefore, the amount of chromatin in the mitotic second chromosome which other workers have called euchromatin is reduced and the number of breaks which the euchromatin receives in proportion to its length is increased. This would indicate that the euchromatin of the second chromo-

some breaks with a frequency 2.6 times the frequency with which heterochromatin breaks. However, if it be assumed that the chromatin between the secondary constriction and the centromere does not break, then euchromatin is breaking with a frequency only 0.3 times that of the remaining heterochromatin. Or if, as suggested by MULLER and GERSHENSON (1935) for the X, the more proximal region of heterochromatin is likewise a block or blocks of chromatin not subject to internal breakage, then the breakage in the remaining heterochromatin between the blocks must be very much more frequent, length for length, than in the euchromatin.

Since the regions on both sides of the centromere in the mitotic chromosome are not represented by more than one band each in the salivary chromosome, there is a possibility that the centromere itself may not be present in these highly specialized chromosomes. However, it seems improbable that these components of the normal chromosome are gathered in a "chromocentre" of the salivary cells in the way FROLOVA (1936) claims.

It is shown in this paper that there is a region in the mitotic chromosomes adjacent to the centromere of the second chromosome which is not represented by more than one band in the salivary gland chromosome; and that the bulk of the heterochromatic region of the second salivary gland chromosome is formed not from the heteropycnotic material of the mitotic chromosome but from a non-heteropycnotic region distal and adjacent to the heteropycnotic chromatin.

SUMMARY

A secondary constriction appears on the left arm of the second chromosome of *Drosophila melanogaster* in mitotic nuclei but is not differentiated in the salivary gland chromosome.

The region between the constriction and the centromere (forming about one-fifth of the left arm of the mitotic chromosome) is shown by analyses of translocations not to contribute more than one band to the formation of the salivary gland chromosome. A similar region is shown to be present in IIR.

Another region is described for the left arm of the mitotic second chromosome, adjacent and distal to the constriction. This more distal of the two regions forms the bulk of the heterochromatin of the salivary gland chromosome. It, or a part of it, is represented by another fifth of the left arm of the mitotic chromosome.

Terminal heterochromatin could not be conclusively demonstrated in the single case studied by this method.

In the light of these data and data already known for the X, BAUER'S data (1939) are analyzed and the relative frequency of breaks due to X-rays in the euchromatin and in the heterochromatin is discussed.

ACKNOWLEDGMENT

I wish to express my appreciation to DR. B. P. KAUFMANN for suggesting the problem and for helpful criticisms. The conclusions, however, are entirely those of the author.

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ABSTRACTS OF PAPERS PRESENTED AT THE 1941 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

COLD SPRING HARBOR, NEW YORK, AUGUST 27–29, 1941
DALLAS, TEXAS, DECEMBER 29–31, 1941

B. P. KAUFMANN, *Secretary*
Carnegie Institution of Washington
Cold Spring Harbor, New York

ABBE, E. C., and PHINNEY, B. O., University of Minnesota, Minneapolis, Minn.: *The action of the gene dwarf$_1$ in the ontogeny of the stem in maize.*——Maize plants which are d_1d_1 in constitution have a lower rate of increase in length of stem and mature more slowly than do normal plants. The number of stem units is the same, but the stem units are shorter at maturity in the d_1d_1 plants so that these plants are dwarf in stature. Stem unit eight, being reasonably representative, was used for further intensive study. Since the shoot apices of dwarf and normal plants are similar in size, form and cellular constitution, while the subsequently formed young stem unit of the dwarf has shorter cells than does the normal, it was concluded that the threshold of action of d_1d_1 is at just that stage in ontogeny when the stem unit becomes recognizable as a morphological entity. Coupled with the lower growth rate of the dwarf is the slower rate of cell division throughout the ontogeny of stem unit eight. During the earlier phases of stem growth the cells of the dwarf are slightly shorter than are those of the normal plants when stem units of the same length are compared, while in the later phases they are somewhat longer, indicating that the rate of cell enlargement in the dwarf differs from that in the normal. Thus the state of tension between two major morphological phenomena——rate of cell division and rate of cell enlargement——differs in the dwarf from that in the normal. At maturity, however, stem unit eight is longer in the normal because there have been more cytokineses and cell enlargement has proceeded more rapidly than in the dwarf. The action of d_1 in the ontogeny of the stem is thus a multiple action, influencing a series of morphological phenomena which in turn affect stature.

ATWOOD, SANFORD S., U. S. Regional Pasture Research Laboratory, State College, Pa.: *The multiple oppositional alleles causing cross-incompatibility in Trifolium repens.*——In order to determine the number of oppositional alleles (S_1, S_2, S_3, etc.) causing self- and cross-incompatability in two series of plants (17 and 10 individuals, respectively) which had been selected for breeding purposes, each individual was crossed with a homozygous plant (S_1S_1). In all but one of the 27 resultant F_1 progenies, two intra-sterile, inter-fertile groups were found. From this it can be concluded that 26 of the 27 parents each carried two different alleles and the other might have since only five F_1 plants were tested. Within each series all F_1 groups were mated diallely, and each F_1 group

or its heterozygous parent was crossed as a female with six plants, homozygous respectively for six other alleles (S_2-S_7). Since all F_1 plants bore the common factor S_1, an incompatible mating between any two indicated that the two alleles being tested were the same. Likewise, failures with the homozygous plants indicated identical alleles. In order to be certain that failure to set seed was due to identical alleles, all incompatible matings were repeated two or three times, and the reciprocal crosses were made. All possible matings were accomplished and about 47 percent of the diallel crosses were tested reciprocally. In this way 34 alleles from the 17 plants in the first series were tested among themselves, and each was tested with the seven in homozygous plants. Of these 41 alleles, 34 (83%) proved to be different. In the second series, 25 (96%) of the 26 alleles were different.

BAKER, MONTEE R., and IBSEN, HEMAN L., Kansas State College, Manhattan, Kan.: *Two modifiers of self (S) and white-spotting (s) in guinea pigs.*—— The dominant modifiers *Pi-1* and *Pi-2* (pigment inhibitors) affect the expression of both *S* (self) and *s* (white-spotting). One of these modifiers alone has no visible effect on a self (*S*), but when both are present there is a varying number of small white spots on the head along the mid-dorsal line, even though the animal is *SS*. White-spotted animals (*ss*) not carrying the modifiers (and therefore *pi-1 pi-1 pi-2 pi-2*) usually have a large amount of pigment. So far as can be determined at present, the two modifiers are duplicates. The chief evidence for this consists in the fact that two animals may be quite similar in regard to the location and the amount of their white-spotting, and yet by breeding tests it can be shown that if one carries *Pi-1* the other carries *Pi-2*. When both *Pi-1* and *Pi-2* are present in a *ss* individual the pigment is greatly reduced, resulting in an animal that is all white except for some small pigmented spots located anteriorly and usually covering the ears and eyes. Only one animal supposedly of the composition *ss Pi-1 Pi-1 Pi-2 Pi-2* has been produced up to the present. The apparent cases of incomplete dominance of *S* over *s* can be explained in our animals by the presence of both *Pi-1* and *Pi-2* in the heterozygote (*Ss*).

BEADLE, G. W., and TATUM, E. L., Stanford University, Calif.: *Genetics of biochemical characters in Neurospora.*——From material X-rayed prior to meiosis, single ascospore cultures are grown on a medium to which is added, in the form of yeast extract, as many as is practicable of the substances normally synthesized by the organism. Such strains are subsequently tested for loss of synthetic abilities by transferring them to "minimal" media containing inorganic salts (ammonium nitrogen), a carbon source (sugars, starch or fat have been used in various tests), and biotin (the one required growth factor that cannot be synthesized by the normal strains). Induced loss of synthetic ability is indicated by failure to grow normally on such minimal media. Among the mutants obtained in this way are: 1) one unable to synthesize vitamin B_6 (pyridoxine), 2) one unable to make the thiazole half of vitamin B_1 (thiamine)

and 3) one unable to make *para*-aminobenzoic acid. Each of these differs from the normal by a single gene, and each is made indistinguishable from normal by supplying it with the particular substance that it cannot synthesize. These facts are consistent with the assumption that each of the genes involved is concerned with the control of one and only one specific chemical reaction. Other mutants, not yet investigated as regards inheritance, are characterized by loss of ability to synthesize a growth factor different from any vitamin known to the authors, loss of ability to utilize fat as a carbon source, and in a number of other ways. The general approach offers promise both as a method of learning more about how genes control specific processes and as a means of studying biochemical processes as such.

BEASLEY, J. O., and BROWN, META SUCHE, Texas Agriculture Experiment Station, College Station, Texas: *Asynaptic plants of Gossypium and their polyploids.*——In the F_2 of *Gossypium hirsutum* by *G. barbadense*, American upland by Sea Island cotton, fertile and sterile plants were found in a ratio approximating fifteen fertile to one sterile. At first metaphase the fertile plants had the normal 26 pairs of chromosomes while different sterile plants averaged six to nine pairs of chromosomes. Doubling the chromosome number in the sterile plants failed to restore normal chromosome pairing and fertility.

BEATTY, ALVIN V., University of Alabama, University, Ala.: *Mitotic periodicity in leaves.*——The examination of leaf tissue from fifteen families of plants, including 20 species of herbs, vines, shrubs and trees, has revealed a rhythmic cycle in mitotic division which is very similar in all species. In general each one exhibits two maxima periods of increased activity at twelve hour intervals occurring at about three in the morning and three in the afternoon, the morning one being the most active, and two minima periods, which are quite constant for all species and follow soon after the maxima, occurring at about seven in the morning and five in the afternoon. In most species the rise from a minimum to a maximum is characterized by minor fluctuations in mitotic activity.

BERNSTEIN, MARIANNE E., Carnegie Institution of Washington, Genetics Record Office, Cold Spring Harbor, N. Y.: *The incidence and Mendelian transmission of mid-digital hair in man.*——To determine the mode of inheritance of the distribution of mid-digital hair on the human hand, data were collected by BURKS and analyzed by BERNSTEIN and BURKS on parents and offspring and on sibling pairs of high school age. DANFORTH's finding that with very few exceptions a child will have no more fingers affected than its more hairy parent was confirmed.——A hypothesis of monomeric inheritance was offered: that genes for the more affected phenotypes are dominant and allellic with respect to genes causing less affected phenotypes. The validity of this hypothesis was tested on the family and sibling data by statistical methods with good results. ——It was also observed that the proportional frequency of the more and of the less affected phenotypes differs for groups of diverse European origin.

For a group of Italian derivation less mid-digital hair was observed than for a group of Irish derivation, and still more mid-digital hair was encountered for a group of North European-Germanic derivation.

BISHOP, D. W., University of Pennsylvania, Philadelphia, Pa.: *Cytological demonstrations of chromosome breaks soon after X-radiation.*——First spermatocyte prophase stages of Orthoptera are used to demonstrate X-ray induced chromosome breaks. Three minutes after irradiation breaks in one of the four diakinetic chromatids can be demonstrated (*Chortophaga viridifasciata* treated with 540 *r*). In *Melanoplus bivittatus*, fixed six minutes after irradiation (270 *r*) many cases of breaks are found in one of the two visible threads of pachytene chromosomes and in one of the chromatids of diakinetic chromosomes. Within this time interval no cases of simultaneous breakage of two visible threads occurs. However, within thirty minutes (270 *r*) two-thread as well as one-thread breaks occur in diakinetic chromosomes. A few double breaks were found within eight minutes at 540 *r*. These results are evidence for an almost immediate effect of the irradiation in contrast to a delayed effect. However, the breakage reaction seems to be a gradual rather than an instantaneous event, judging from the longer time required for two-thread breaks at the same dosage. Chromosome fragments are found in spermatogonial and spermatocyte anaphases within 30 minutes after irradiation. The point of breakage in prophase chromosomes is at random. In normal and in treated cells neither the pachytene nor the diakinetic chromosomes are in contact with one another; if the breakage mechanism is the same as that leading to inversions and translocations, the evidence supports the breakage-first as opposed to the contact hypothesis.

BISHOP, D. W., University of Pennsylvania, Philadelphia, Pa.: *Sensitivity of spermatocyte chromosomes to aberrations induced in the embryos by X-radiation.*——Gonads were removed from adult and late nymphal grasshoppers (*Circotettix verruculatus*) which had been irradiated (150 to 750 *r*) as embryos still within the chorion. In the first meiotic anaphase there are chromosome alterations, not found in any other mitotic stage, and not included among the usual cytogenetic changes. These aberrations affect one or both of the diad components of the tetrad, and include constrictions, attenuations, and breaks. They are invariably located between the point of spindle attachment and the nearest region of association of the arms of the homologs ("chiasm"). The second spermatocytes thereby produced frequently contain large duplications and deficiencies and are inviable. All of the cells within an affected cyst have been derived from a single treated cell by at least seven divisions. Yet no abnormalities in the previous spermatogonial, diakinetic, or first spermatocyte metaphase chromosomes predict the aberrations that will occur at meiotic anaphase. Comparisons with chromosome rearrangements involving reciprocal translocations (multiples), inversion-crossovers (dicentrics and acentrics), and small deletions, demonstrate that some other mechanism of chromosome alter-

ation pertains. The distribution of aberrations is non-random, concerning the regions affected within particular chromosomes as well as different chromosomes within a cell. A large proportion of aberrant chromosomes show homologous breaks, symmetrically placed, in the two members of a tetrad. The conclusion is drawn that the X-ray effect is general and the chromosomes become fragmented at points of natural weakness.

BOGART, RALPH, MUHRER, M. E., and HOGAN, A. G., University of Missouri, Columbia, Mo.: *The physiology and inheritance of a hemophilia-like abnormality in swine.*——A hemophilia-like condition, characterized by prolonged bleeding, has been observed in swine. Hemorrhages from slight injuries, or no visible injury, are frequently fatal. The coagulation time of whole blood and of plasma from "bleeder" hogs is greatly extended but once formed the clot retracts in normal time. Blood from "bleeder" hogs is normal in quantity of prothrombin, fibrinogen, calcium and formed elements. Either tissue juices or platelets will cause blood from "bleeder" hogs to clot in a normal manner. This indicates that blood from "bleeders" is deficient in an available source of thromboplastin. The abnormality first expresses itself at about two months of age and becomes more severe with increasing age. The data, which include 84 matings and 585 offspring, indicate that the defect is due to a recessive gene. There is considerable variation in severity of the character which indicates that modifying genes influence the degree to which the "bleeder" gene expresses itself. No evidence for sex-linkage has been obtained.——(Contribution from the Departments of Agricultural Chemistry and Animal Husbandry, Mo. Agri. Exp. Sta. and the Bureau of Animal Industry, U.S.D.A. Mo. Agri. Exp. Sta. Journal Series No. 790. Aided by a grant from the John and Mary R. Markle Foundation.)

BREHME, KATHERINE S., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *A survey of the Malpighian tube color of the eye color mutants of Drosophila melanogaster.*——The color of the larval Malpighian tubes of certain eye color mutants was found by BEADLE to differ from the bright yellow characteristic of the wild type. The tube color of 25 mutants was reported by BEADLE, and of eight others by BRIDGES, CURRY and SCHULTZ. Because of the usefulness of this character in classifying larvae for experimental purposes, observations have been made of 66 additional mutants, virtually completing the list of factors affecting eye color. No direct relation has been found between the amount of pigment in the eye and in the tubes. Alleles which have a similar effect upon eye color appear to have a similar effect upon tube color, although the safranin and vermilion alleles may offer exceptions to this generalization. Alleles which have a different effect upon eye color (the white locus) affect tube color in varying degrees, and dark eye color is not necessarily accompanied by dark tube color. Two useful chromosomal aberrations are readily classifiable in the larva: T (2; 3) Pale (white tubes) and In (3L) persimmon (almost white).

BRINK, R. A., and COOPER, D. C., University of Wisconsin, Madison, Wis.: *Somatoplastic sterility as a function of the endosperm genotype.*——Following the mating, *Nicotiana rustica* ♀ by *N. tabacum* ♂, most of the seeds collapse although a few attain a germinable condition. This combination is intermediate between *N. rustica* selfed, which is fully fertile, and *N. rustica* ♀ by *N. glutinosa* ♂ in which all the seeds fail early. The two classes of hybrid seeds are similar in that (a) endosperm growth is retarded, (b) pronounced hyperplasia of the nucellus occurs and (c) the integumentary cells distal to the vascular bundle do not differentiate into conducting elements. With reference to (a) and (b) above, however, the hybrid seeds differ in degree. The endosperm-nucellus volume ratios at the two-celled proembryo stage, for example, are as follows: 1.47 for *rustica* selfed; .72 for the *tabacum* hybrid; and .51 for the *glutinosa* hybrid. Similarly, the percentage of seeds in which the endosperm is occluded by nucellar overgrowth at the 8- to 16-celled embryo stages is zero, 22 percent and 76 percent, respectively. All the *tabacum* hybrid seeds continuing growth beyond early stages show the endosperm in direct association with the integument through the nucellar gap at the chalazal end. Growth of these seeds is at the expense of the integument, the cells of which tend to become depleted of their contents.

BRITTINGHAM, WM. H., U. S. Regional Pasture Research Laboratory, State College, Pa., and The University of Maryland, College Park, Md.: *The nature and extent of variation in Kentucky bluegrass as criteria for apomictic seed formation.*——A nursery of 10,066 plants was established from 115 parental plants of *Poa pratensis* L. Seed set under bag varied from none to over 80 percent, with 65 percent of the plants giving over 60 percent seed set under parchment bag. Self-pollinated progenies were available from 87 parental plants. The following average figures were obtained: germination 80.3 percent, polyembryony 7.0 percent, survival in the field 86.3 percent, and among single seedlings 14.8 percent, and among twin seedlings 17.1 percent deviated from their respective maternal types. The highest value obtained for polyembryony was 31.6 percent, the highest for variability 65.5 percent. The lowest value for survival was 27.5 percent and was found in the progeny giving the highest value for variability. No significant correlations were found between variability and source of material, seed set under bag, and germination. A barely significant negative correlation existed between polyembryony and variability. A highly significant negative correlation was found between survival and variability. Morphological and cytological studies have indicated that plants from both single seedling and twin seedling sources arise from 1) apomictic development of reduced eggs ("haploids"), 2) fertilization of reduced eggs by reduced pollen ("diploids" by amphimixis), 3) fertilization of unreduced eggs by reduced pollen ("triploids"), and 4) apomictic development of an unreduced egg derived from a cell of the nucellus by apospory and resulting in matroclinous offspring ("diploids" by apomixis). The results of this work are not inconsistent with the conclusion that techniques of selection, strain building, and intra-

and interspecific hybridization are theoretically applicable to breeding problems in Kentucky bluegrass.

BRUMFIELD, R. T., Yale University, New Haven, Conn.: *Cell-lineage studies in root meristems by means of X-ray induced chromosome rearrangements.*——Certain types of chromosome rearrangements induced by X-rays are passed on through subsequent cell division to all the descendants of the affected cell. Thus chromosome changes induced by X-rays offer a means of "tagging" a single cell and determining the kind and extent of tissues derived from it. This paper reports results obtained by the use of this method in studying cell lineage in root meristems.——Germinating seed of *Vicia faba* and *Crepis capillaris* were X-rayed, allowed to grow for three weeks after radiation, then primary root tips were fixed and sectioned transversely. Cytological observations revealed that many of the root meristems thus treated were chromosomal chimeras, both normal and rearranged complements (usually unequal translocations) being found in the same root. All chimeras so far observed have been sectorial ones. In most of the chimeras of both Vicia and Crepis the sector having the rearranged complement represents about one-third of the root and includes root cap, epidermis, cortex, endodermis, and central cylinder. This indicates that in these roots a single cell may give rise to all the above tissues and that the whole root develops from but few cells at the extreme tip. Sectorial chimeras would not be expected if the meristem consisted of "histogens" as described by HANSTEIN.

BUSHNELL, RALPH J., University of Connecticut, Storrs, Conn.: *Incompatible matings in inbred families of the bean weevil.*——The common bean weevil, *Acanthoscelides obtectus*, provides excellent material for the study of fertility of inbred families as the infertile eggs collapse soon after being deposited. In a series of 151 matings, the individuals comprising the matings being derived from field infested beans, 7.28 percent of the males were involved in infertile matings. Offspring from 66 of the original matings were brother × sister mated and from a total of 449 such matings, 21.2 percent of the males and 20.7 percent of the females were involved in incompatible matings. Actually, only 368 males were used as some males were tested with two or more of their sisters. Tests of the latter type showed that some males were compatible with all sisters with which they were tested, other males being compatible with some but not all of their sisters, while a few males were compatible with none of their sisters. In many instances, the members of an incompatible pair had been observed in coition. Incompatible matings occurred in 33 of the 66 families selected for the first generation of inbreeding. However, 11 of the 33 families not showing incompatibilities in the first generation of inbreeding did show them in the second. Of the families in which incompatible matings occurred in the first generation of inbreeding, 28.6 percent (89 out of 311) of the matings were infertile, while the second generation of brother × sister matings produced 23.9 percent (82 out of 343) incompatibilities. Of the families in

which incompatibilities became evident for the first time in the second generation of inbreeding, there were 23.85 percent incompatible matings (26 out of 109) in that generation.

CASPARI, ERNST, Lafayette College, Easton, Pa.: *Genetic and environmental factors influencing testis color in Ephestia kühniella.*——Two loci are known to influence testis sheath pigmentation in Ephestia. The series a^+-a^k-a diminishes the quantity of pigment in testes, as well as in eyes and other organs, by controlling the release of a diffusible substance necessary for pigment formation. A gene pair *Rt-rt* controls the quality of the pigment formed in the testes, but not in the eyes. *Rt* testes are brownish violet, containing evenly distributed sepia colored granules of equal size. *rt rt* testes are red or pink, containing red pigment granules of unequal size and sometimes pigment diffuse in the cells. The strength of pigmentation of the testes is furthermore influenced by the hatching order, the first hatching animals having more heavily colored testes than the later hatching ones. This applies to *RtRt*, *rtrt* and *aa* animals. The eyes of *aa* animals do not show any influence of hatching order. It is concluded that this factor influences testis pigmentation through some process other than the formation of a^+ substance.

CHASE, ELIZABETH BROWN, and CHASE, HERMAN B., Department of Zoology, University of Illinois, Urbana Ill.: *Inheritance of shape of vaginal orifice in the mouse.*——The vaginal orifice was examined at the time of introitus in two inbred strains of mice and in the offspring of crosses between them. In one strain a large oval vagina was found in 100 percent of the cases (102); in the other, a small round vagina was found in 179 and oval in 5 females. In the F_1, oval vagina shape was found in 41, round in one female. In the F_2 there were 91 oval and 29 round. In the back-cross to the round type, there were 30 oval and 27 round produced. These results would seem to indicate a single factor affecting vagina shape, showing dominance of the oval type.

CHASE, HERMAN B., Department of Zoology, University of Illinois, Urbana, Ill.: *A mutation toward normal eyes in the anophthalmic strain of mice.*——In the sixth generation of inbreeding a female appeared with a normal left eye and a nearly normal-sized right eye, a condition much nearer normality than was ever recorded in this strain. This female mated with her completely eyeless brother produced offspring indicating that she was heterozygous for a factor for normal eyes. From this mating two sublines have been selected, one with 98 percent anophthalmics and the other with 94 percent normals. Crosses with other strains have shown that *Ey* (normal) is dominant over *ey* (eyeless). Crosses between these two sublines have shown no dominance but a complete range of expression from normal through completely eyeless. A matroclinous tendency is shown, however, in the shifting of the median of this range toward normality with a normal-eyed mother.

CLANCY, C. W., University of Oregon, Eugene, Ore.: *Development of eye colors in Drosophila melanogaster: Further studies on the mutant claret.*——Evidence for the existence of ca^+ substance has been re-examined. Unpublished data supplied by Professor G. W. BEADLE of Stanford University along with results of experiments carried out by the writer show, 1) that the type of non-autonomous development undergone by a wild-type optic disc when grown in a claret host is non-specific and occurs in other hosts known either to lack v^+ hormone or to contain it in reduced amount as compared to wild type, 2) that wild-type optic discs grown in claret hosts contain less brown pigment than wild-type control implants, 3) that wild-type optic discs grown in hosts of claret constitution can approach full wild-type pigmentation very closely when additional v^+ hormone is supplied, and 4) that by a variety of tests the mutant claret exhibits less v^+ hormone activity than wild type. In view of these results the original basis for the postulation of ca^+ substance is no longer tenable. A method for the extraction and measurement of the eye color pigments of Drosophila has been devised and preliminary values obtained in a series of measurements on wild type, claret, and double-recessives of claret with vermilion, cinnabar, and brown. These show that the mutant gene claret acts in such a way as to reduce the quantity of the red pigment to about 17 percent, and the brown pigment to about 28 percent of that present in wild type.

CLARK, FRANCES J., Connecticut Agricultural Experiment Station, New Haven, Conn.: *Preliminary investigations in Zea mays of the germination capacity of pollen with aberrant nuclei.*——Maize plants which are homozygous for the recessive mutant *dv* (divergent spindle) produce microspores whose normal chromosome complement is distributed among several to many micronuclei. Usually a return to the normal nuclear condition follows the first division of the microspore. Occasionally, however, the distribution of the chromosome complement among several nuclei may persist resulting in multinucleated vegetative or generative cells or uni- or multinucleated cells with no division of the cytoplasm. Such male gametophytes may develop normal appearing pollen grains. To determine whether these grains are capable of germination, pollen of *dv/dv* plants was placed on small pieces of silks. After 30 minutes the silks were fixed, stained in aceto-carmine and cleared with chloral hydrate. Of the 287 pollen grains examined, one had two nuclei and two had three nuclei in the vegetative cell. Two sperm cells were also present in each of these three grains. The pollen grain with two nuclei in the vegetative cell had commenced germination. Of 17 pollen grains containing *only* one or two nuclei (no sperm nuclei present), eight had produced normal appearing germ tubes which had penetrated the silk hairs. It is concluded that pollen germination does not require a normal morphological organization of the pollen grain (one vegetative cell and nucleus and two sperm cells with one nucleus each) nor the inclusion within a single nucleus of all the chromosomes of the vegetative cell.

CLELAND, RALPH E., Indiana University, Bloomington, Ind.: *Report on the*

analysis of segmental arrangements of Onagra complexes.——The segmental arrangements of a considerable number of Onagra genoms are being analyzed, and the present status of this work is summarized. Considerable progress has been made since the last report. The smear method is now being used almost exclusively in the determination of chromosome configurations. A few examples of such preparations are exhibited.

COLE, LEON J., and FINLEY, HAROLD E., University of Wisconsin, Madison, Wis.: *The production of somatic mutations in the pigeon with X-rays.*—— The exhibit shows flight feathers from ash-red pigeons of genotype $B^A B$. Feathers on the right panel are from the right wing; on the left are the corresponding feathers from the left wing. In the top row are feathers plucked before any treatment; they show relatively little flecking. Immediately after plucking the empty follicles in the right wing were exposed to 750 r and 1000 r of X-rays; those in the left wing received no treatment. In the second row are the feathers regenerated from these follicles. It is evident that the amount of flecking has been greatly increased in the treated feathers. That a permanent change has been produced is indicated by the tendency to reproduce the flecking pattern in three subsequent feather-generations from these same follicles (rows 3, 4 and 5) without further treatment. This increase in flecking may be attributed to mutations produced in the feather germ by the X-rays, either by change of the B^A gene to B or b, or by the production of a deficiency in that region; the present experiment is not decisive as to which.

CROUSE, HELEN V., University of Missouri, Columbia, Mo.: *Translocations in Sciara: Their effect on chromosome behavior and sex determination.*——In *Sciara coprophila* maps have been made of the four salivary chromosomes and the points of breakage in ten translocations localized. Salivary chromosomes B and X of *S. reynoldsi* have been mapped. Studies on four translocations place the Puff locus on chromosome B, the sex-linked recessive, yellow, on X. B and X are the two rod-shaped chromosomes in metaphase. In *S. coprophila* the translocation studies identify salivary chromosomes X, II, and III as the three rods in metaphase and associate the Wavy, Fused, and Curly linkage groups with these three chromosomes; map sections 12, 22, and 34 mark their respective centromere ends. Chromosome IV is the V-shaped chromosome; Dash is located on IV.——The translocation studies support the identification of the precocious chromosome as the sex chromosome of Sciara. When the X is translocated reciprocally with autosomes in *S. reynoldsi* and *S. coprophila*, only one of the translocation chromosomes exhibits precocious behavior in the secondary spermatocyte. This chromosome has been demonstrated to be the one with the X centromere in three separate instances. In *S. coprophila* there are no factors distal to map section 10 B which control the precocious behavior. In all cases the chromosome which exhibits precocious behavior during spermatogenesis is the one eliminated from the soma and germ line during embryogeny. Males which transmit an X-translocation ordinarily produce

only daughters. The male embryos do not hatch, presumably because of the unbalanced chromosome complement in their soma subsequent to chromosome elimination. The female embryos develop in spite of the unbalanced complement in their nuclei prior to the elimination process.——The translocations were produced in strictly monogenic *S. coprophila*. Females heterozygous for translocations involving any one of the autosomes are monogenic. Females heterozygous for each of the five X-translocations regularly produce offspring of both sexes. The "exceptional" males in all cases have rudimentary sterile testes; males with a small ovary and a sterile testis have been observed. The "exceptional" females produce sons and daughters. The intact X chromosome appears to be essential to the monogenic condition.

CUMLEY, R. W., IRWIN, M. R., and COLE, L. J., University of Wisconsin, Madison, Wis.: *Genic effects on serum proteins.*——Although no differentiation of the serum proteins of Pearlneck (*Streptopelia chinensis*) and Senegal (*S. senegalensis*) was possible by direct precipitin tests with Pearlneck antisera, it was found that Pearlneck serum could be definitely distinguished from Senegal serum in tests with the "reagent" produced by absorbing certain anti-Pearlneck sera with Senegal serum. Three such reagents were tested with the sera of 14 backcross birds, obtained from mating, to Senegal, species hybrids and two backcross hybrids from the cross of Pearlneck and Senegal. A definite segregation of serum antigens specific to Pearlneck was observed, showing that the antigens specific to Pearlneck are gene-determined.——Furthermore, a comparison of the presence or absence of antigens, specific to Pearlneck, of the blood cells (also gene-controlled) and serum, respectively, of these backcross hybrids showed that such antigens of the serum might be present either in the presence or absence of demonstrable cellular antigens. This segregation shows that the antigens specific to Pearlneck in the serum and cells, respectively, are not produced by the same genes, and only a loose linkage, if any, obtains between them.——The results of this and previous investigations show beyond reasonable doubt that the species-specific qualities of the serum proteins are determined by gene action and suggest that the total protein complex is likewise determined by genes.

DEMEREC, M., HOLLAENDER, ALEXANDER, HOULAHAN, M. B., and BISHOP, M., Carnegie Institution of Washington, Cold Spring Harbor, N. Y., and National Institute of Health, Bethesda, Md.: *Effect of monochromatic ultraviolet radiation on Drosophila melanogaster.*——Abdomens of male flies of yellow-white and wild type stocks were pressed between quartz plates and irradiated with measured quantities of monochromatic ultraviolet radiation of the following wave lengths: 2280, 2650, 2937, 3050, 3130 and 3300 Å. The flies were analyzed for sex linked lethals by *ClB* method. Some sterility was induced by all wave lengths tested, shorter wave lengths proving to be more effective than the longer ones. A sharp break in the effectiveness took place between 3050 and 3130 Å. Sterility is apparently due to injury produced in

the tissues by radiation which penetrates the abdominal wall. Energy required to produce sterility at 2280 Å is about 2.1×10^6 ergs per cm^2 and the sterility limit is so low that only 1 percent of lethals can be obtained. At 3130 Å energy of 83.5×10^6 ergs per cm^2 can be easily applied since it sterilizes only about 50 percent of the treated males. Such dosage induces about 5.5 percent lethals. Yellow-white flies showed higher sensitivity to injury than wild type flies.——For 3130 Å data are available indicating that the genetic effect increases with the dosage applied, but that the interference of secondary factors induces a great deal of variability in successive experiments. This is probably caused by the absorption of radiation by tissues which happen to cover the testes during irradiation. In occasional experiments the percentage of lethals obtained was as high as 50 percent indicating the possibility that ultraviolet may produce mutations at a very high rate.——In one experiment with 2650 Å one translocation among 116 treated sperm was observed. Additional 762 tests of sperm from males which had been given similar treatment did not disclose any translocation. None was observed among 1073 sperm obtained from males treated at 3130 Å with energy which produces 5.5 percent lethals.

DEMEREC, M., KAUFMANN, B. P., and SUTTON, E., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Genetic effects produced by neutrons in Drosophila melanogaster.*——Oregon-R, wild-type males were treated with neutrons using the Institution's electrostatic generator, with a lithium target. During the treatment, which lasted several hours, flies were kept at constant temperature and humidity, and shielded from beta rays by one-fourth inch of lead. Dosage measurements were made with a 25 r Victoreen chamber and are expressed in arbitrary n-units.——Two groups of males were treated with about 1500 n-units, and two with about 2000 n-units. Part of the males from each group were mated with *ClB* females in order to determine the frequency of induced X-chromosome lethals. The remaining males were mated with wild-type females, and the salivary gland chromosomes of the F_1 female larvae were analyzed for breaks.——The lower dosage produced $23/1221 = 1.88$ percent lethals, and the higher dosage gave $30/1068 = 2.81$ percent. From X-ray studies of the Ore-R line by Demerec, and from Timoféeff-Ressovsky's and Zimmer's studies on the production of lethals by neutrons it is inferred that in this experiment n-units can be transferred into r-unit equivalents by multiplying values by a factor of about two. Cytological analysis showed for the lower dosage 18.43 ± 2.43 percent altered sperms and 46.66 percent breaks per total sperms, and for the higher dose 20.2 ± 2.33 percent altered sperms, and 53.5 percent breaks. Comparable values for equivalent treatment with X-rays are: 18.82 ± 1.60, 49.23; 29.8 ± 3.12, and 85.58 percent respectively. These results indicate that the frequency of breaks induced by neutrons does not differ appreciably from the frequency induced by an equivalent dose of X-rays. Breaks are also distributed at random throughout the chromosomes.

DODGE, B. O., The New York Botanical Garden, New York, N. Y.: *A herit-*

able factor complex for heterocaryotic vigor in Neurospora.——A new bright cadmium-yellow dwarf race of Neurospora which has a very low growth rate is non-conidial. Races C_4 and C_8 of *Neurospora tetrasperma* both produce conidia and they are of opposite sex. They are stable races so far as growth rates and production of conidia under standard cultural conditions are concerned. When nuclei of the dwarf race and those of C_4 are brought together in the same cytoplasm to give a heterocaryotic mycelium, this mycelium grows very much more vigorously, two or three times as fast as does that of race C_4 alone and the amount of conidia produced is increased manyfold. The dwarf race reacts with race C_8 which is of the same sex, in the same way, increasing the growth rate and the production of conidia very strikingly. When the dwarf race is crossed with race C_4 and new races are obtained by germinating the f_1 ascospores, some of these new races show the same bright yellow non-conidial dwarf characteristics. When nuclei from these are allowed to mingle with nuclei of either C_4 or C_8 in a common cytoplasm the same heterocaryotic vigor is manifested. Furthermore, some of the new races which are unisexual show very vigorous growth with production of large amounts of conidia. Since the nuclei of their mycelia must be haploid and homocaryotic, the vigor shown is simply due to favorable new combinations of genes now included within the same nuclear membrane. Heterocaryotic vigor, true hybrid vigor and individual haploid inherited vigor are to be distinguished.

Eigsti, O. J., University of Oklahoma, Norman, Okla.: *A comparative study of the effects of sulfanilamide and colchicine upon mitosis of the generative cell in the pollen tube of Tradescantia occidentalis (Britton) Smyth.*——Concentrations of each drug within the range of 1:1000 and 1:10,000 induced mitotic abnormalities. Greater concentrations of each drug inhibited pollen tube formation. Consequently the generative cell remained apparently inactive mitotically. There are several noticeable effects of colchicine upon mitosis of the generative cell; *viz.*, unseparated daughter chromosomes scattered in the pollen tube, daughter chromosomes relegated to the same nucleus, and disturbed metaphasic stages which preclude regular sperm formation. Sulfanilamide also induces mitotic irregularities such as: interrupted metaphasic stages; irregularly formed nuclei; and disintegrated chromosomal material. The maximum number of nuclei attainable in pollen tubes growing in a medium containing colchicine is six (one nucleus for each pair of chromosomes); whereas, more than six nuclei frequently appear in the pollen tubes of sulfanilamide preparations. Eight hours after the pollen was planted onto the medium containing sulfanilamide small globular bodies appeared in the plasm of certain pollen tubes. A duplication of chromosomes is not the usual result of treatment with sulfanilamide; however, colchicine usually causes duplication of chromosomes in the nucleus. This feature of duplication in the presence of colchicine and lack of duplication in the presence of sulfanilamide is the notable difference between the effects of these drugs upon mitotic phenomena of the generative cell of the pollen tube of *Tradescantia occidentalis*. These studies indicate that

the genetic consequences of treatments of meristem with sulfanilamide might possibly differ from those of colchicine in which case polyploidy is the usual result from meristem treatment.

FANKHAUSER, GERHARD, and CROTTA, RITA, Princeton University, Princeton, N. J.: *The frequency of spontaneous aberrations of chromosome number among larvae of the newt, Triturus viridescens.*——During the past four years, a systematic investigation has been made of the chromosome numbers of larvae of the common newt, *Triturus viridescens*. The counts were made in whole-mounts of tailtips amputated at an early larval stage. The larvae all developed from eggs laid in the laboratory from November to May, by pituitary-stimulated females. Several triploid larvae (FANKHAUSER, 1938; GRIFFITHS, 1941) and a pentaploid larva (FANKHAUSER, 1940) were discovered during the first three years. The past year has added other cases of triploidy and pentaploidy, as well as several other types of numerical aberrations. Sufficient material has thus accumulated for a preliminary estimate of the frequencies of the various aberrations in this species.——A total of 1074 larvae have been classified as to chromosome number so far. 1056 were diploid (22 chromosomes), 10 triploid, 1 tetraploid, 3 pentaploid, and 1 haploid. One of the three remaining larvae possessed a complement of 24 chromosomes, one was a haploid-diploid chimaera, while the tailtip of the third contained both diploid and hypertriploid cells. Of particular interest are the three pentaploid larvae and the single haploid which is probably the first case of spontaneous haploidy to be described among vertebrates. Less extensive studies of larvae of other salamanders (*Triturus pyrrhogaster, Eurycea bislineata*) have shown that similar spontaneous chromosome aberrations occur in these two species also.

GILES, N. H., and NEBEL, B. R., Yale University, New Haven, Conn., and New York Agricultural Experiment Station, Geneva, N. Y.: *An analysis of the intensity factor in X-ray induced chromosomal aberrations in Tradescantia.*——In a series of experiments developing microspores of Tradescantia were given equal total doses (308 roentgens) of X-rays at widely different intensities, from 1.3 to 1200 r/min. At the 4–5 day period following irradiation smears were made for an analysis of the percentages of translocations (dicentric and ring types) and of interstitial deletions——small acentric rings according to RICK and SAX (isodiametric fragments in the terminology of NEBEL, WILSON and MARINELLI) induced during the resting stage at the various intensities. At 50 r/min. and above (150, 600, and 1200 r/min.) the percentage of aberrations in both classes remains approximately constant——there is little or no intensity effect. At lower intensities (20, 10.3, 5.2, 2.5, and 1.3 r/min.) the percentage of aberrations in both classes decreases progressively, but not linearly with decreasing intensity, until at 1.3 r/min. the number of aberrations per cell is somewhat less than half that found at intensities of 50 r/min. and higher. These results are in accord with the hypothesis that the chromosomal aberrations types studied result from two independent potential breaks,

and that the reunion of broken ends occurs during the resting stage within a certain time following the initial breakage. When this time is exceeded restitution occurs. Other interpretations involving second order processes may also require consideration.

GOOD, CHARLES M., JR., and NABOURS, ROBERT K., Kansas Agricultural Experiment Station, Manhattan, Kan.: *Genetic problems of the grouse locust Tettigidea parvipennis Harris.*——There are 19 genes for color pattern, and two for wing and pronota lengths, now known in *T. parvipennis*, most of them being dominant. These are located on at least four of the seven pairs of chromosomes of the species. Though there are 11 dominant genes on one of the pairs of chromosomes no crossing over has been observed. With fewer numbers of genes on any other pair of chromosomes to work with still no crossing over is known. Apparent cases of linked genes are known but crossing over has not occurred. Most of the patterns can be distinguished in any combination of genes so there is apparently a large number of allelic genes on each of the pairs of chromosomes. One of the patterns (not sex-linked) does not show in the female. There are three different genes producing analogous cuffings or bandings of the hind femora. These differ from each other in primary and secondary characters. *T. parvipennis* are also now being studied cytologically.

GOPAL-AYENGAR, A. R., Department of Botany, University of Toronto, Toronto, Canada: *Structure and behaviour of meiotic chromosomes in Gymnosperms.*——By a technique which involves pretreatment of the material before fixation, with NaCN 2^{-5} Mol. for 45 seconds to one minute and subsequent staining by the use of the Feulgen nucleal reaction or with acetocarmine, it has been possible to unravel the coiling systems of the meiotic chromosomes in species of Pinus, Taxus and Ginkgo. The number of gyres of the major spirals of particular chromosomes can be determined with certainty and its frequency is variable among and between bivalents. Generally speaking, there is a direct relation between chromosome length and the number of gyres. Reversals of coiling occur on either side of the centromere. There is undoubted evidence of a minor coil in the I metaphase and anaphase chromosomes of all the species investigated. The chiasma frequency of Ginkgo is between 1.8–2.5. Meiotic aberrations include inversions, which give rise to bridge configurations at I anaphase. In *Pinus nigra* v. *austriaca* up to 5 percent bridges were found. There is very little sterility in the pollen grains.

GOPAL-AYENGAR, A. R., Department of Botany, University of Toronto, Toronto, Canada: *Structural hybridity in Scilla species.*——Cytological examination of several species of Scilla and related genera has shown a high degree of chromosomal abnormality. These include asynapsis, univalence at meiosis, inversions, deletions and translocations, microcyte formation and ameiosis. The frequencies of bridges and fragments are very high in *Scilla siberica* (n=6). Approximately 39 percent of the cells show bridges ranging from one

to six at I anaphase. Nearly 8 percent of the cells possess two bridges in a cell (including those formed as a result of complementary crossovers in single and successive or overlapping inversions). There is also definite evidence of reciprocal and disparate crossing over in successive inversions. About 1.2 percent of the cells have three bridges and .1 percent show 4, 5 and 6 bridges respectively. Of the remaining, 15 percent of the cells have only fragments at I anaphase. There is a decrease in the number of bridges at II anaphase, though not considerably. In Bellevalia species there is a slight increase in the frequency of bridges at II division thus indicating the previous existence of loops at I anaphase. The difficulties that arise in the quantitative estimation of the coefficient of hybridity and hybridity equilibrium are discussed in the light of the chromosome behaviour of these species.

GORDON, MYRON, New York Aquarium, New York Zoological Society, N. Y.: *Dominant and recessive responses of the Sd factor in natural and domesticated fish populations.*——The gene *Sd* for a black-spotted dorsal fin has been known to be present in natural populations of *Platypoecilus maculatus* since 1867. *Sd* appears to be sex-linked dominant when tested with *sd* fish taken from its own natural habitat in the Rio Papaloapan, Mexico. When wild *Sd* fish are tested with *sd* fish of domesticated, aquarium-reared stocks, the spotted dorsal character does not appear in F_1. An extremely small number of *Sd* fish have been recovered in F_2, but the ratios indicate no simple Mendelian basis for the results. When *Sd P. maculatus* from natural populations are hybridized with three different *Xiphophorus hellerii* stocks: albino, red, wild, all the F_1 hybrids display the spotted dorsal character in an exaggerated manner. In some hybrids the condition leads to the first, and in others to the second, state of melanosis of the dorsal fin, similar in some respects to that produced by the *Sp* factor on the body proper.

GORDON, MYRON, New York Aquarium, New York Zoological Society, N. Y.: *A third primary factor, Sd, for melanomas in hybrid fishes.*——It has previously been shown that when the macromelanophore factor, *Sp*, for black spotting, or *N* for black band of *Platypoecilus maculatus*, is combined with *Sp* and *N* modifiers, *A* and *B*, of *Xiphophorus hellerii* the hybrids develop melanomas. When these hybrids are backcrossed to *X. hellerii*, the severity of the melanomas among the offspring of the next generation is intensified, often evoking the development of melanomas in day old fishes. A third factor for macromelanophores, the cells that evoke melanomas, has been discovered among the wild population of *P. maculatus* in Mexico. Owing to the fact that the macromelanophores are confined mainly to the dorsal fin of this new variety it has been called "the spotted dorsal" factor, *Sd*. When an *Sd P. maculatus* is mated with *X. hellerii*, the dorsal fin of the hybrid is almost entirely infiltrated with macromelanophores; this results in a partial breakdown of the fin and denotes the second state of melanosis. When fertile hybrids are backcrossed to *X. hellerii* the condition of melanosis is intensified and appears

much earlier. The pathological behavior of the macromelanophores, evoked by *Sd*, appears to be similar to that of *Sp* and *N* under hybridization.

GOWEN, JOHN W., Iowa State College, Ames, Iowa: *On the physiological basis of inherited disease resistance.*—Our investigations have established six inbred strains of mice differing markedly in typhoid resistance. Two strains are highly susceptible, two fairly susceptible and two have great resistance; the differences between the extremes being of the order of a thousandfold. Search for physiological factors which might account for these differences in inherited resistance has shown the level of the blood cells to be of real significance. The strains differ in the numbers of both red and white cells, as well as in the proportions of the different leucocytes. The differences in numbers of red and white cells are correlated with the resistance and susceptibility. The resistant strains show more of each type of cell than those of intermediate resistance; strains of intermediate resistance have a greater number than strains which are susceptible. Changes in the proportions of the leucocytes have less significance than greater total numbers of cells.

HERTEL, ELMER W., Wartburg College, Waverly, Iowa: *Studies on vigor in the rotifer, Hydatina senta.*——Bisexual inbreeding of the rotifer, *Hydatina senta*, resulted in a variety of strains having different degrees of vigor. There, however, was found to be a uniformity in the vigor within a given strain as was demonstrated by the rate of parthenogenetic reproduction. By selection and bisexual inbreeding for three generations nearly pure homozygous weak strains were obtained. The original vigor was restored to a marked degree by selection and crossbreeding of the weak strains. Multiple factors are probably responsible for the variation in vigor. About 4000 female rotifers, which produced more than 94,000 young, were raised and recorded during these investigations. Four unrelated strains of the rotifer were used. The vigor of the parental stocks, the inbred generations, and the cross-bred generations was determined by recording the number of young females produced by a mother. Culture conditions were kept uniform throughout all of the experimental work. Buffer solutions were used to maintain a pH of 7.2 in the culture solutions at a temperature of 16° to 18°C. A flagellate, Polytoma, was added daily as food for the rotifers. Chlamydomonas was fed instead of Polytoma for the production of males in cross-breeding.

HINTON, TAYLOR, and ATWOOD, K. C., Columbia University, New York, N. Y.: *A comparison of the specificities of terminal adhesions of salivary gland chromosomes in two strains of Drosophila.*——The two-by-two adherence of chromosome ends is non-random in salivary chromosome preparations of two wild type strains (Oregon-R and Swedish-b) of *D. melanogaster*. The frequency with which the various combinations of adhesions occur differs significantly between the two strains. The Oregon-R data was presented in an earlier report by HINTON and SPARROW (1941). In 107 cases recorded for Swedish-b, the

combinations occur as follows: 2L-3L—36; X-3L—18; X-2L—15; 2L-3R —11; 2L-2R—10; 2R-3L—9; 3L-3R—8; X-3R—6; 2R-3R—3; X-2R—1. The F_1 of the cross between the two wild type strains has a pattern of specific adhesions which is a close fit with the Oregon-R pattern, indicating a kind of dominance of the Oregon-R pattern over that of the Swedish-b. These data indicate that at least three groups of factors are contributing to the nonrandomness of the adhesions. The groups of factors are tentatively defined as: chromosome length, a non-specific stickiness, and four or more different specificity factors. When unsmeared nuclei were studied from total mount preparations of glands, 42 out of 50 showed free ends. This indicates that the nonrandomness is not due to a differential pulling apart of chromosome ends from a common mass during the process of smearing. The arrangement of chromosomes in the nucleus is found to have no correlation with the adhesion pattern.

HOLLANDER, W. F., Palmetto Pigeon Plant, Sumter, S. C.: *A sexually dimorphic factor in the pigeon.*——In previously published studies it has been shown that the mutant "Faded" is dominant to wild-type coloration, and sex-linked. The phenotypes of the "Faded" female (hemizygous) and the heterozygous male are indistinguishable. Recently the homozygous male has been produced and tested; the phenotype is markedly different from that of the female or heterozygote, even in late embryonic stages. Pigmentation is reduced to a trace, and the nestling down is considerably shortened. The homozygous males show normal vigor and fertility. The "Faded" factor therefore is an excellent basis for auto-sexing strains; preliminary matings indicate that perfect accuracy in distinguishing sex can easily be obtained, at least by the time the squabs are two weeks old.

HOVANITZ, WILLIAM, California Institute of Technology, Pasadena, Calif.: *Variable frequencies of a dominant color gene in a wild population.*—The sex-limited, dominant, homozygous lethal, white female polymorphic form of *Colias eurytheme* (Lepidoptera) appears to occur in various frequencies in wild populations; the normal color is orange. Counts made of adults at various intervals throughout the season of an apparently continuous breeding population (at Mono Lake, California) from early spring until winter have disclosed a wide range in saturation of the gene at that locality. Extreme frequencies in 1941 range from 60 percent in May to 13 percent in late July to 52 percent in early October in a series of eight approximately 2½ week interval samples. The fluctuations form a reasonably smooth curve of variation and nearly all samples are either statistically significant from adjacent samples or samples one farther removed. The adaptational mechanism allowing a gene to exist in such high concentrations (with a lethal disadvantage) in competition with an apparently satisfactory "wild-type" is not yet apparent; likewise, the reasons for the fluctuations remain obscure.

HUESTIS, R. R., University of Oregon, Eugene, Ore.: *Gene interaction in*

Peromyscus.——Among random tests for linkage in Peromyscus a cross between flexed tail and silver agouti produced only one double recessive mouse in one thousand F_2 young but no commensurate paucity of the F_2 wild-type class. The single individual has sired more than one hundred test cross young. The data make it probable that the flexed tail-silver agouti combination is usually lethal, somewhat probable that the genes are linked.

HUNT, H. R., HOPPERT, C. A., and ERWIN, W. G., Michigan State College, East Lansing, Mich.: *Heredity in rat caries.*——This experiment (started early in 1937) is designed to discover whether heredity is partially responsible for susceptibility to dental caries in rats. The caries-producing diet consists of 66 percent of coarsely ground rice, 30 percent of whole milk powder, 3 percent of alfalfa leaf meal, and 1 percent of sodium chloride. The rats are placed on this diet at 35 days of age and are observed fortnightly for cavities in the lower molars. The time required to produce the first cavity is the criterion of susceptibility. A susceptible and a resistant strain are being developed. Very close inbreeding is being used in each line. Breeders are selected in accordance with three criteria: 1) the time they require to develop caries, 2) the time required by their siblings, and 3) ancestry. The phenotype alone is unreliable as a guide in selection. For example, a 25 day male mated with 25–31 day females gave offspring with a mean of 60 days. The same male mated with 72–86 day females produced offspring averaging 61 days. Nearly 2000 rats have been studied. The current averages of sibship means are as follows. Susceptible line: 2nd generation, 57 days; 3rd, 43 days; 4th, 37 days; 5th, 32 days; 6th, 29 days; 7th, 38 days; 8th (incomplete), 28 days. Resistant line: 2nd generation, 116 days; 3rd, 142 days; 4th, 168 days. Thus hereditary differences are evident. The ranges of the incomplete 6th generation resistants and 8th generation susceptibles show little if any overlapping.

HUTT, F. B., and MUELLER, C. D., Cornell University, Ithaca, N. Y.: *On the linkage relations and manifestation of polydactyly in the fowl.*——Tests of several mutations for linkage with the recently-discovered linkage group containing duplex comb (*D*) and multiple spurs (*M*) revealed that a dominant gene for polydactyly, *Po*, belongs in that chromosome. The relationship of the three genes is *D*—28—*M*—33—*Po*. The expression of polydactyly is quite variable and it is completely suppressed in many heterozygotes. Diallel crosses of the same *Popo Mm* females with two different *popo mm* males showed that modifying genes preventing the appearance of polydactyly in heterozygotes also inhibit the manifestation of multiple spurs. Thus, in progeny of one of these males, 49 percent showed *Po* and 48 percent *M*, while in chicks from the other male only 16 percent were polydactylous and 29 percent showed multiple spurs. The expectation in each case was 50 percent. Similar suppression of both characters was observed in other matings. Linkage of *Po* with *M* as measured in over 500 gametes in the first of these matings was 33.5 percent. In the second mating, in which both characters were suppressed, the amount of ap-

parent crossing over between the two genes was 56 percent. *Po* causes duplication of one or more phalanges of the first digit, while *M* induces centers of ossification in scales not normally ossified. Although these two processes are not identical, they have some common features and it is perhaps to be expected that both would be suppressed by the same modifiers, since the areas affected are in close proximity.

IRWIN, M. R., and CUMLEY, R. W., University of Wisconsin, Madison, Wis.: *Suggestive evidence for duplicate action of genes.*——The antigens of blood cells are more or less primary products of their causative genes, although a few examples of presumed interaction in the production of cellular characters are known. Thus hybrids between Pearlneck (*Streptopelia chinensis*) and Ring dove (*S. risoria*) possess "hybrid substances" not present in their respective parents.——Several different characters (d-1, d-2 · · · d-11) particular to Pearlneck, have been obtained in progeny of backcross to Ring dove.—— A part of the hybrid substance has always been present with the Pearlneck character d-11; another, but different part, with d-4. Other parts of the hybrid substance have been associated with the Pearlneck characters d-1, d-2, d-3, d-7, d-9, and d-10. However, the hybrid substances correlated with each of these six different characters of Pearlneck are indistinguishable by immunological tests, but they are definitely different from the other two hybrid components. Thus the "hybrid substance" of these species hybrids is divisible into at least three structurally different antigens.——In contrast to the complete association observed of two different parts of the hybrid substance with Pearlneck characters d-4 and d-11, respectively, only a loose linkage, if any, exists between the gene or genes responsible for each of the six different Pearlneck characters (d-1, d-2, d-3, d-7, d-9 and d-10) and those for the hybrid substance associated with each of these characters.——Two alternative explanations may be offered for these findings. 1) A single chromosome of Pearlneck, carrying one or more genes which by interaction with a gene or genes of Ring dove produce a third part of the hybrid substance, has usually been present in each of the various backcross individuals possessing the respective chromosomes carrying genes for the six different characters peculiar to Pearlneck. This explanation, however, appears highly improbable. 2) There are one or more genes on each of several chromosomes of Pearlneck which, by interaction with a gene or genes of Ring dove, produce indistinguishable and probably identical effects. The genetic data suggest this as the more probable interpretation.

IVES, P. T., Amherst College, Amherst, Mass.: *Allelism and elimination of lethals in American populations of Drosophila melanogaster.*——Lethal second chromosomes extracted from wild populations of *D. melanogaster* were tested against each other for allelism and against lethals occurring in the laboratory. In 1176 crosstests of 49 wild So. Amherst, Mass., lethals, the rate of allelism was 0.43 ± 0.20 percent. In 1200 crosstests of 50 wild Winter Park, Fla., lethals, the rate was 0.33 ± 0.17 percent. In neither case does the rate of elimination of

lethals per generation approach the apparent lethal mutation rate per generation. In 2261 crosstests of 27 lethals occurring in the laboratory, both to each other and to 54 wild lethals, and of 10 Belfast, Me., wild lethals to 45 So. Amherst ones, the rate of allelism was 0.31 ± 0.12 percent. The lack of significant differences between these rates suggests that the alleles found in the wild samples represent separate mutations of the same locus, in each case, rather than common parentage of the lethal chromosomes.

JAAP, R. GEORGE, Oklahoma Agricultural Experiment Station, Stillwater: *Modifiers of the sex-linked gene for barred feathers and sex identification in newly hatched chickens.*——Stages in reversal of dominance produced by modifiers of the Z^B gene are demonstrated by means of skins from day-old chickens. At this time one Z^B gene produces no visible effect on "wild-type" down coloration, either in the heterogametic female or in the heterozygous male. This phenomenon is responsible for positive sex identification of chicks within a true breeding color pattern. Modifiers which intensify pigmentation in the down cause reversal of dominance, the extent of reversal being largely dependent on the degree of melanism. The form with a maximum amount of melanic pigment is the only condition of modifiers recognized in The American Standard of Perfection. Color differences between the heterogametic female and the homogametic male are indistinct in this form. When the basic down color is made less intense than that of the "wild type" lack of sufficient pigmentation causes the effects of the Z^B genes to become indistinct and sex identification inaccurate. Positive sex differences, possible only with the combination of modifiers which has been designated as "wild type" are useful for research in sex physiology as well as providing a useful convenience to the industry. The skins included in the exhibit represent all degrees of melanism in the "gold" and "silver" phenotypic patterns.

JEFFREY, EDWARD C., and HAERTL, EDWIN J., The Biological Laboratories, Harvard University, Cambridge, Mass.: *Chromosomal phenomena in fertilization.*—This important field has remained practically unworked so far as the internal organization of gametic chromosomes is concerned on account of the inadequate technique for the fixation of chromosomal structures imbedded in tissue. As the result of a number of years of experiment a medium has finally been developed which apparently brings about reliable fixation of the internal structures of all chromosomes. Due to this situation it is now possible to outline the details of chromosomal organization in fertilization. Investigations have been carried on on Lilium, Tradescantia, and Erythronium. The latter has proved particularly favorable. Two reversely coiled chromatids are present in both the fusing sperm and egg nuclei (which are in a prophase condition). The too commonly held view that only one chromatid is present in reproductive chromosomes is consequently inaccurate. The spiremes of both nuclei are continuous and they become united end to end to form the prophase of the first division in the fertilized egg. The divisions of the egg and subsequent mitoses

in the embryo show the same chromosomal organization as in the reproductive nuclei. The same situation is also found in the divisions of the endosperm and of the gametophyte. In meiosis precisely similar conditions are found, namely a continuous spireme, breaking up into chromosomes which contain two reversely spiralled chromatids. These facts appear to make necessary a fundamental revision of the accepted views in regard to fertilization and meiosis. It is now apparently clear that the meiotic chromosomes differ in no respect from any others except in number.

KAMENOFF, RALPH J., City College, New York, N. Y.: *A cytological study of the embryonic livers (16–18 days) of normal and flexed-tailed (anemic) mice.*——An embryonic anemia of the flexed-tailed mice as early as 14 days' gestation has been recorded. Since the liver is the seat of erythrogenesis in late embryonic life, reaching its maximum activity about the 17th day, an examination of the liver including counts of various types of cells was undertaken. A greater total density of cells was found in the 16th and 17th day normal livers than in the flexes, while the 18th day normal is less dense, due to the greater number of larger vacuolated liver cells. Less erythrogenesis is taking place in the 18-day normal livers. There are also some differences in the types of cells present. In the 16th and 17th days the flexed-tailed mice show a higher percentage of the earlier erythrogenic cells than do the normals. This may be due to a delay in erythrogenesis in the flexes. Further study of the earlier stages is being made.

KAUFMANN, B. P., and DEMEREC, M., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Sperm utilization in Drosophila melanogaster following single and multiple inseminations.*——It has been found by actual count that the number of sperms transferred in a single copulation by the four to five day old, wild-type male approaches 4000. In succeeding copulations the number is gradually reduced until after a few consecutive matings no sperm is transferred. The amount of sperm delivered in a single copulation is only sufficient to ensure fertilization of all eggs laid during the first few days by the average female. Subsequently the percentage of sterile eggs increases, until toward the end of the egg laying period practically all eggs deposited are unfertilized.——A measure was made of the ability of sperms of wild-type, Curly/Glazed and plexus brown speck males, when used in all possible mating sequences, to take precedence in fertilizing the eggs of *px bw sp* females. Curly and Glazed offspring from these crosses were slightly more frequent than wild-type; the *px bw sp* represented only about one-sixth of the total. Similar proportions were found among the offspring of *px bw sp* females, which were permitted to lay their eggs in the same bottle, after each mated with only one of the types of males available. Such similarity suggests that differences in frequency of various classes of offspring following polyandry are referable to differential viability of the different types of embryos and larvae, and not to selective fertilization.

KEELER, C. E., and KING, H. D., The Wistar Institute, University of Pennsylvania, Philadelphia, Pa.: *The taming effect of coat color genes in the Norway rat.*——Modification profiles are exhibited for various coat color genes in the Norway rat displayed on a fairly uniform residual hereditary background, detailing their specific effects on anatomy and behavior. An analysis of popular laboratory strains of rats is presented to show that most of these strains are tame by virtue of the effects of their coat color genes on behavior rather than due to the modifying effects of captivity.

KIMBALL, R. F., Johns Hopkins University, Baltimore, Md.: *A gene affecting the manner of swimming in the ciliate protozoan, Euplotes patella.*——A gene affecting the manner of swimming in the ciliate protozoan, *Euplotes patella*, has been found. The dominant allele of the gene determines that the animals swim in a wide spiral with very little forward motion so that they practically move round and round in a circle. The recessive allele determines that the animals swim in a narrow spiral with considerable forward motion. The expression of the character is considerably influenced by nutritive and other conditions so that animals of the homozygous recessive genotype, if in very poor condition, tend to the circular type of movement. If animals with a dominant allele are subjected to a short temperature shock of over 38°C, they swim for a short time in a narrow spiral. Individual variability also occurs so that occasional animals of one genotype may swim in the manner characteristic of the other. However, large cultures under good conditions can almost invariably be classified with certainty.

KING, ROBERT L., State University of Iowa, Iowa City, Iowa: *Inheritance of melanism in Melanoplus differentialis.*——A small percentage of *Melanoplus differentialis* collected in the Great Plains and adjacent regions are black with a few yellow markings. A mating between a single black female, found in 1930, and wild type males gave an approximate ratio in F_2 of 3 type:1 black. The sex ratio among the black individuals was 1:1. In the summer of 1938 a relatively large number of black individuals were collected as nymphs in eastern Nebraska and eastern South Dakota. Black individuals mated to black give all black; black individuals mated to wild type give all wild type. Various matings of such F_1 hybrids gave the following results: inbreeding of F_1 gave 449 type to 133 black in F_2; back crossing F_1 males to black females gave 222 type to 236 black; backcrossing F_1 females to black males gave 241 type to 285 black. There were approximately equal numbers of males and females in all classes of offspring. These data are consistent with the assumption that the gene for the black color phase is recessive to wild type, not sex-linked.

LAANES, T., and MACDOWELL, E. C., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Screw-tail, a new mouse mutation.*—This mutation is most conspicuously indicated at birth by the tail, which is usually tightly coiled. Later this corkscrew is opened out and leaves the tail bent into various

angular patterns with thickenings at the angles; similar kinks are found in the spine. Growth and development are markedly retarded. At birth screw-tail mice are smaller than their normal litter mates and about 24 percent are found dead, compared with 8 percent; many more die before weaning. Their small size and general ill-favored condition persist. Breeding is very rare; the only productive matings of screw-tail mice have been with screw-tail-producing normals (14 normals to 15 screw-tails) and most of the young were found dead.——Screw-tail first appeared from two sib mothers of the 50th brother by sister generation of the CSH-Bagg albino strain; these two families included 46n:13 s.t. and 57n:19 s.t. Two other females (sibs of the above two) by the same sib male gave 67 and 76 normals and no screw-tails. Subsequent inbreeding of the normal descendants of these four females gave families with 100 percent normals and other families with ratios approximating 3:1 (23.0% s.t. in a total of 1357). The deficiency of screw-tails in the total is due to males (21.1% s.t.) as the females alone gave 24.7 percent s.t. At birth there is a relative deficiency of males among screw-tail young; the totals show 44 percent males as compared with 49.1 percent males among normal litter mates.

LAMOREUX, W. F., HUTT, F. B., and HALL, G. O., Cornell University, Ithaca, N. Y.: *Genetic selection for low fecundity in the fowl.*——Selection of a strain of White Leghorns genetically capable of only low egg production was started at Cornell in 1914 and has been continued to the present time. With mass selecton, as practised until 1934, average annual production (365 days after first egg) in 1914, 1915, and 1917 was 97, 99, and 83 eggs respectively, but only once from then until 1935 was average production less than 102 eggs. Beginning in 1935, the breeders were selected on the basis of the performance of progeny or siblings, and the production of pullets hatched that year was 79 eggs, the lowest in the records of the experiment. Since that time the records have been tabulated only to 500 days of age. However, most hens of the low-fecundity strain cease laying before that age and the change therefore results in only a slight reduction in the egg record—in 1935 less than three eggs. That this sudden decline in production was a direct result of the change in methods of selection is shown by the fact that average production during the succeeding five years (1936–40) was 68, 58, 59, 67, and 40 eggs. In contrast, fowls bred for economic characters, including high fecundity, lay from 170 to 180 eggs in the 500-day period and most of them lay many more before ovulation ceases. The difference in these strains under similar environmental conditions demonstrates the extent to which modern records of high productivity are dependent upon the genetic constitution.

LANDAUER, WALTER, Storrs Agricultural Experiment Station, University of Connecticut, Storrs, Conn.: *A new mutation of fowl affecting the axial skeleton, especially in the tail region.*——Description of a mutation which appeared in a stock of White Leghorn fowl. The most common expression resembles that of the well-known rumpless mutation and its modifications: the caudal vertebrae,

the external tail and the uropygial gland are partly or entirely absent. Completely rumpless animals are relatively rare. In incompletely rumpless animals the morphological expression is somewhat different from that of the intermediate rumpless fowl which have been described earlier. In addition, lordoses, kyphoses, extra ribs and other abnormalities of the axial skeleton occur rather commonly in this stock. Breeding data will be discussed.

LAWSON, C. A., Wittenberg College, Springfield, Ohio: *The mechanism controlling production of aphid types.*——The germaria of female aphids can be differentiated on the basis of appearance and size into four types. 1) Gamic female, which produce gamic eggs and are found in gamic females. 2) Male producing, which produce male eggs and are found in some wingless parthenogenetic females during the gamic phase of the cycle. 3) Wingless parthenogenetic female, which produce parthenogenetic female eggs and are found in female-producing wingless parthenogenetic females. 4) Winged parthenogenetic female, which produce female eggs and are found in winged parthenogenetic females. When gamic-parthenogenetic female intermediates have both gamic and parthenogenetic female germaria the parthenogenetic female germaria are always the male producing type. The embryos contained in these intermediates are usually male. If any are female they are usually gamic female. Winged-wingless parthenogenetic female intermediates may have germaria of any type except all gamic female. It is assumed that a single sex substance is produced during the gamic phase of the cycle which causes wingless female type germaria to become the male producing type, which then produce male eggs. The same sex substance in winged parthenogenetic females has no effect on the winged female type germaria which continue to produce female eggs, but the sex substance does effect these eggs causing them to develop into gamic females. The production of winged and wingless types is explained by assuming that embryonic wingless parthenogenetic female type, male producing type and gamic female type germaria produce a wing inhibitor, while winged parthenogenetic female type germaria and males, which have no germaria, do not.

LEWIS, E. B., California Institute of Technology, Pasadena, Calif.: *The Star and asteroid loci in Drosophila melanogaster.*——Further studies of the two rough-eyed mutants, dominant Star (*S*, 2-1.3) and recessive asteroid (*ast*), formerly called Star-recessive (S^r), in *D. melanogaster* show that asteroid is located 0.02 map unit to the right of Star. Sixteen wild-type cross over products between the two loci have been recovered as well as three cases in another experiment of the complementary type having Star and asteroid on the same chromosome. From each of the latter cases, asteroid, identical with the original asteroid as far as can be detected, has been derived by crossing over with a normal chromosome. A comparison of *S ast*/++ and *S*+/+*ast* reveals a striking position effect: whereas, *S ast*/++ has normal wings and roughened eyes like *S*+/++, *S*+/+*ast* has extensively interrupted wing

venation and extremely small rough eyes. When these two closely linked mutants are on opposite chromosomes they act phenotypically as allelomorphs. The finding that Star and asteroid are located in the 21 E1–2 doublet of the salivary gland chromosomes suggests a possible basis for this phenomenon in as much as such doublets have been interpreted as natural repeats in the chromosomes. A salivary gland chromosome analysis shows no departure from normal in the case of *S*, *ast*, *S ast* and the wild-type cross over complement to *S ast*.

LINDEGREN, CARL C., and LINDEGREN, GERTRUDE, University of Southern California, Los Angeles, Calif.: *X-ray and ultraviolet induced mutations in Neurospora.*——The uninucleate thin-walled spores (spermatia) of *Neurospora crassa* were treated by ultraviolet and X-rays cultured individually in separate test tubes and compared with an adequate number of controls. The variants induced were true gene mutations, for when mated with mycelia of opposite sex, factors differentiating the variations segregated at meiosis. A considerable number of the mutations, from both sources, reverted. Reverse mutations are much more easily brought into expression and have an opportunity to obtain supremacy in the fungi because the nuclei are haploid and the thallus is structureless and coencytial. A reverse mutation in any one of the nuclei in the thallus may change the phenotype. It is possible that the so-called "temporary changes" found by EMMONS and HOLLAENDER are likewise mutations which reverted. Fifty percent of the spermatia treated with X-rays were killed and over 99 percent of those treated with ultraviolet were killed at the dosages used. A considerable number of the X-ray variants gave evidence of the presence of chromosomal aberrations, while this was not true of any of the ultraviolet variants.

LINDSTROM, E. W., Iowa State College, Ames, Iowa: *Inheritance of seed longevity in maize inbreds and hybrids.*——Embryo longevity of maize varies to a marked degree and is definitely heritable. Germination tests with hundreds of inbred lines and F_1 crosses of 5–12 year-old seed, stored at room temperature in Ames, showed germination percentages ranging from 0 to 90 percent. Some inbred lines failed to live in storage more than 3–5 years. Experiments under controlled conditions of temperature (40°–50°–60°C.) and of humidity (0–30–60–90 percent by H_2SO_4 solutions in desiccators) were devised to hasten respiration or reduce longevity. Hybrid seed of long-lived by short-lived parental inbreds exhibited the long-lived condition, often with significant differences in reciprocal crosses. The number of genes involved is not always large because one-generation selfs in varieties (Krug and a Reid's Yellow Dent strain) gave significant differences in longevity of seeds. Curves of temperature and humidity effects on the respiration or aging of dormant inbred and hybrid maize seeds are presented.

LUCE, WILBUR M., University of Illinois, Urbana, Ill.: *Effects of formalin*

upon facet number in the bar alleles of Drosophila melanogaster.——HINSHAW, in press, studied the effects of formalin upon bar eye. Her preliminary observations indicate a reduction in facet number is brought about by the treatment. In the series of experiments reported upon here, bar-infrabar(BB^i), bar(B), infrabar(B^i), and double bar(BB) all derived recently from one original strain(BB^i), were raised on food to which there had been added from 0.3 cc to 1.2 cc of formalin to every 100 cc of water used in making the banana-agar-yeast food. Thirty to 35 eggs were placed in each of the vials used. All flies were raised at 27°C except during the egg collecting period the latter being done at 24°C. Critical facet counts of flies raised in the low concentrations are not yet complete but preliminary observations give no evidence of any effect on facet number here. The percentage of eggs hatching and the general conditions of development were better than in the control vials. In the highest concentrations of formalin the percentage of hatch, the number of pupae produced, and the number of adults emerging were all somewhat less than in the case of the controls indicating that the formalin was having a lethal effect. Here too, the length of time necessary to complete development was also increased. The mean facet number was reduced in all cases by the treatment with the highest concentrations of formalin, the percentage of reduction as compared with the controls varying from 24.47 percent in bar males to 13.84 percent in bar-infrabar males.

MAINLAND, GORDON B., University of Texas, Austin, Texas: *The Drosophila macrospina group.*——The *macrospina* group falls into three subdivisions: *Drosophila macrospina macrospina* extending from central Texas eastward into Florida and northward into Ohio; *D. macrospina limpiensis* being found in west Texas, New Mexico, Arizona, Utah, and Sonora; and *D. subfunebris* known only from the vicinity of Pasadena, California. In all tests attempted *macrospina* and *limpiensis* strains cross readily and produce normal numbers of progeny. F_1 progeny from *macrospina* ♀ × *limpiensis* ♂ are fully fertile while the reciprocal cross yields fertile females and sterile or semi-sterile males depending upon the strains employed. In general this sterility decreases as the distance between the strains of the two subspecies decreases. No *macrospina-subfunebris* crosses have produced progeny with the exception of central Texas strains; a small percentage of central Texas *macrospina* ♀ × *subfunebris* ♂ crosses produce progeny while the reciprocal cross fails. All *limpiensis-subfunebris* crosses attempted have yielded progeny in at least one direction. As the geographical distance between the *limpiensis* strains and *subfunebris* decreases, the matings go more readily. All F_1 males from *subfunebris* × *limpiensis* or *macrospina* are sterile while their sisters are fully fertile. In the F_2 progeny from interspecific backcrosses, not all of the females are fertile and less than 1 percent of the males are fertile. The *macrospina* group is a chain of strains extending from one coast to the other. On the basis of genetics, cytology, and systematics, the two end members are distinct species; but gene interchange from one end of the chain to the other is possible through intermediates.

McNUTT, C. W., and SAWIN, P. B., Brown University, Providence, R. I., and Ohio State University, Columbus, Ohio: *A genetic study of the pattern of the vena cava inferior in the rabbit.*——Variations in the pattern of the vena cava and its tributaries occurring in 2300 rabbits from seven unrelated, and in some cases inbred, families and from crosses between them are separable into 29 distinct categories. The conclusion that these variations are primarily genetic is drawn from the following evidence: 1) The distribution of normal and atypical venae cavae in the several families is significantly different both quantitatively and qualitatively. 2) In the F_1 of crosses between predominantly normal families with those possessing a high incidence of abnormality, the normal type is the more frequent. 3) In the F_2 and in successive backcrosses segregation is apparent, but tendencies for the distribution to be continuous and intermediate between those of the parental generations involved are obvious throughout. These observations coupled with the facts that the "recessive" segregates do not breed true, but through selection can be used to establish a strain almost 100 percent atypical, seems to indicate a multifactorial interpretation. Ontogenetically the variations represent the persistence of portions of the bilaterally symmetrical postcardinal, supracardinal and cardinal collateral embryonic venous systems, which in normal development successively undergo atrophy between the fetal fourteenth and sixteenth days. It is tentatively suggested that these variations are the result of functional adaptation of the vascular system to meet altered conditions produced by hereditarily controlled changes in differential growth.

MICKEY, GEORGE H., Louisiana State University, Baton Rouge, La.: *Polysomaty and polyploid gametes in Romalea microptera.*—Polyploidy occurs in the male germ cells of Romalea as well as in the sheath around the follicles. Varying numbers of chromosomes up to octoploid are found in spermatogonial plates, but they are nearly always multiples of the haploid. Although most of the spermatozoa are normal haploid, a surprising number of polyploid sperms are found in otherwise apparently normal testes. Diploid, triploid, tetraploid and even pentaploid sperms occur. The abnormal spermatozoa are most easily recognized at an early stage of spermiogenesis, when the chromatin has lost its staining capacity except the heterochromatic X-chromosome. In spermatids containing the sex chromosome, the number of heterochromatic masses is indicative of the degree of ploidy: haploids have one; diploids, two; etc. Another criterion of ploidy is size; but only cells within the same cyst, and consequently in the same stage of development, may be compared in size. Contrast in size is most striking in mid-spermiogenesis when other criteria likewise are more easily evaluated. Another obvious evidence of ploidy is the number of "basal bodies" or ring centrioles present. A normal haploid spermatid has only a single basal body and a single axial filament, whereas diploid cells have two each; triploids, three; etc. Acrosome size and number of nebenkerns are also increased in polyploid spermatids. Evidence concerning formation of polyploid gametes is found in the fact that diploid spermatids occur in pairs, triploids and tetraploids usually occur singly.

MULLER, H. J., and PONTECORVO, G., Amherst College, Amherst, Mass., and University of Edinburgh, Edinburgh, Scotland: *Recessive genes causing interspecific sterility and other disharmonies between Drosophila melanogaster and simulans.*——The effects were studied of the minor chromosomes of *D. simulans* after their transfer (by a method previously reported) into an otherwise *melanogaster* genotype. Males with *simulans* Y were sterile, like XO males. Y-*sim* suppresses variegation somewhat, but less than Y-*mel*. It undergoes some nondisjunction with attached *melanogaster* X's.——Homozygous IV-*sim*, in *melanogaster*, allows fair viability. But it results in a complex of slight, variable, recessive peculiarities, *e.g.*, flattened form, heavier trident, eye reduction, semi-*simulans* male genitalia. Homozygous IV-*sim* males, unlike females, are sterile; their testes are fairly developed but, as EPHRUSSI observed, their vasa contain few or no spermatozoa. This recessive sterility depends upon a narrowly localized chromatin region, almost certainly one gene, since it appears in compounds of IV-*sim* with IV-*mel* containing a "Minute-IV" deficiency. This result is not due to non-allelic interaction between the "Minute" and the sterility processes, for normal-appearing triplo-IV's having two IV-*sim*'s and one Minute-deficient IV-*mel* are likewise sterile.——Evidence for peculiarities of other genes in IV-*sim* is found in IV-*sim*/IV-*mel* heterozygotes in which the IV-*mel* carries a known mutant gene. Thus, the recessive "cubitus" becomes partially dominant under these conditions, while the dominant "Cataract" becomes nearly recessive. Nevertheless, segregation of IV-*sim* from IV-*mel* seems fairly regular.——The fact that even these minor chromosomes exhibit so many gene differences indicates that the reaction systems producing the similar phenotypes of apparently closely related species may be highly divergent. Hybrid sterility is but one expression of this cryptic divergence, which need not in itself have had a selective value.

MULLER, H. J., and PONTECORVO, G., Amherst College, Amherst, Mass., and University of Edinburgh, Edinburgh, Scotland: *The surprisingly high frequency of spontaneous and induced chromosome breakage in Drosophila, and its expression through dominant lethals.*——The unusually low ratio of daughters to sons of irradiated males having ring X-chromosomes is shown to be mainly due to death of zygotes with affected X's. For, at 4000 *r*, only about 3 percent of females are found to be converted into males by induced loss of an X, whereas the relative number of females is reduced by about 35 percent. Allowing for deaths from X-translocations (some 10 percent), most of this dominant lethal effect on the females must be due to "simple breakage," which in rings must often be followed, in cases which would otherwise be restitutional, by formation of dicentrics, occasioned by twisting of the chromonema or chromonema-pair before re-union. The results show over 75 percent of these sister-dicentrics to be lethal (translocational dicentrics being still oftener lethal). Breaks in non-rings are presumably induced by irradiation as often as in rings, but here the great majority are followed by perfect restitution. Thus at 4000 *r* nearly all offspring contain one or more restituted chromosomes. Nevertheless the lethal losses of non-ring X's are frequent enough (*ca* 5% at 4000 *r*) to show

that a considerable part of the dominant lethal effect of sperm irradiation results from "simple breakage" with sister-dicentric formation. The frequency of spontaneous lethal and viable losses of rings (*ca* 13% and 2.5%) is likewise surprisingly high, and may be due to spontaneous breakage. This probably occurs similarly in non-rings but is followed by perfect restitution. Since so few spontaneous structural changes arise, this breakage would usually occur in pre-spermatozoon stages, allowing prompt restitution.

NEBEL, B. R., WILSON, G. B., and MARINELLI, L., New York Agricultural Experiment Station, Geneva, N. Y., McGill University, Montreal, Canada, and Memorial Hospital, New York, N. Y.: *X-ray dosage curves in Tradescantia.*——Three independent dosage curves have been plotted for the action of X-rays on Tradescantia microsporocytes observed 4–5 days after raying. The first set of exposures was made by G. B. WILSON in Geneva, N. Y. early in 1940 and scored in Montreal in 1941. The second set of data was collected by B. R. NEBEL also in Geneva from plants grown in constant environment. The third set of data was collected from radiation applied to cut heads of Tradescantia at the Memorial Hospital by L. MARINELLI. Within the limits of the three experiments environmental differences were found to be unimportant. The method of radiation is held responsible for the results obtained. At Geneva the radiation was intermittent the *off* period being from 30″ to 90″ with the *on* period of equal or shorter duration. The intensity of the *on* period varied between 50 and 200 *r*/m, 100 *r*/m being the most common intensity used.——The Wilson-Geneva and the Nebel-Geneva data are identical. They were scored independently. For rings and dikinetic translocations above 100 *r* the Geneva data show a straight line (K^1) for the relationship between dose and effect. For isodiametric fragments the independent data of WILSON and NEBEL show a more than linear rise for dose and effect——$K^{spp\cdot 1.5-}$. A more than linear rise ($K^{1.5}$) for rings and dikinetic translocations plotted against dosage was obtained from the Nebel-Memorial Hospital experiment. The same experiment showed a value of K^2 for isodiametric fragments. These results were obtained by applying 110, 200, 300 and 400 *r* with varying intensity and continuous exposure over a total time of two minutes for every application.

NEEL, J. V., Dartmouth College, Hanover, N. H.: *A case of high mutation frequency in Drosophila melanogaster.*——In February, 1941, a long inbred wild type strain of *D. melanogaster*, which until then had been quite uniform, was observed to be throwing a high proportion of phenotypically aberrant individuals. Thus far 50 different fertile mutants, 17 different sterile off-types which were probably genetic in origin, and numerous variants (mostly mosaics) whose aberrant characteristics did not reappear in subsequent generations, have been found among a total of 72,851 flies examined. The mutation rate is thus up approximately 5–10 times over the more commonly observed values. Of the 41 mutations that have been localized as to chromosome, 27 are

sex-linked. These latter include 12 yellows, 2 Notches, 2 singeds, 2 bobbeds, a cut, a carmine, and a lozenge. 1706 X chromosomes, derives from two substrains of the mutable stock, were simultaneously analyzed for the occurrence of lethals and visibles; 2 visibles and 15 lethals were found. The rate of occurrence of lethal mutations ($0.88 \pm 0.23\%$) is thus also high in these substrains. However, 985 X's from a third substrain yielded only one lethal and no visibles. The nature of the factor(s) responsible for the increased mutation rate is being investigated.

NEWBY, W. W., University of Texas, Austin, Texas: *An intersex in Drosophila virilis.*——A dominant mutation, found in a wild strain of *Drosophila virilis* from Texas, causes heterozygous female flies to become intersex. Such intersexes are male-like in that the anal plates are vertical in position and claspers are present. They are female-like in that the vaginal plates are present, but these are always reduced in size or are abnormal in shape. The tergites and sternites are larval in character. There are nine tergites; the posterior three being reduced in size, and there are six sternites, the sixth being represented by a pair of small plates.——Internally, the reproductive organs are primarily female but in all specimens the various parts are reduced, absent, or abnormal. The gonads have the yellow color of the testes but the cells are ova-like.

OLIVER, C. P., and GRAY, ROYAL C., University of Minnesota, Minneapolis, Minn.: *The use of genetic information in the control of hereditary ataxia in a human kinship.*——In a kinship, dominant cerebellar ataxia is traced for five generations. Families of ataxic persons have been large; the sibships in living generations are at least as large as average families of today. From one ataxic person of generation I, four of eight children became ataxic. From one ataxic person of generation II, seven of nine children, ten of fifty-six grandchildren, and one of seventy-one great-grandchildren had developed ataxia at the time our records were completed, and others can be expected in the grandchildren and great-grandchildren as they reach the age of onset. The average age at death for ataxic individuals was thirty-seven in generation II, thirty-eight in III, and thirty-two in IV; the five living ataxic persons in IV had an average age of thirty-four. Ataxic women die earlier than ataxic men. With the cases known for certain in generation IV, the average age of onset of the disease was twenty-four, the range from twenty-one to thirty. In this kinship with the early age of onset, the defect can be eliminated. An ataxic person should have no children; and a person with an ataxic parent should postpone having children until he has passed the age of onset, for all practical purposes thirty in this family, and has remained free of ataxia. Medical and genetic data are published in Minnesota Medicine, May, 1941.

PIPKIN, ALAN, and PIPKIN, SARAH BIDICHEK, Tulane University, New Orleans, La., and North Texas Agricultural College, Arlington, Texas: *Albinism*

in negroes.——A study of pedigrees of albinism in four negro families has been made. Three generations are covered in three of the families and five generations in the fourth family. Eye colors appeared as blue, bluish-yellow, greenish-yellow, and cinnamon-brown in the ten albinos observed. Nystagmus was invariably present, photophobia also. Pupils appeared dark. Strabismus was present in two albinos. Hair color varied from a light reddish color to straw yellow. Head hair was of a fine texture, and in most cases the face and arms were covered with a fine downy hair. Hair samples were taken for microscopic study. Freckles were observed in five adult albinos, absent in four albino children, and absent in one adult. In one adult a skin cancer on the face and arms had developed. In three families, there were five albinos and sixteen dark pigmented children born of normal dark pigmented parents, showing albinism to be dependent upon a simple autosomal recessive gene. No consanguinity was recorded in any of these cases. In the fourth family, albinism appeared in three successive generations. From two marriages of a normal dark negro and an albino negro, three dark and five albino offspring resulted. No consanguinity was admitted, and the spouses were reared in different localities. Therefore unless the spouses were heterozygous for recessive albinism in two successive generations, this family must possess a type of albinism dependent on a dominant gene.

POLLISTER, A. W., and MIRSKY, A. E., Columbia University, and the Hospital of The Rockefeller Institute for Medical Research, New York, N. Y.: *Preparation of a nucleohistone from mammalian organs, and direct demonstration of its nuclear origin.*—From liver and other organs (kidney and spleen) there has been extracted a nucleoprotein of high phosphorus content (3.8%), all phosphorus being present in the form of desoxyribose nucleic acid (shown by positive Feulgen and Dische reactions). The protein is a histone. Preparations are highly viscous, and show birefringence of flow, indicating rod-shaped molecules. The protein is soluble in 1.0 M NaCl, and insoluble in 0.14 M NaCl (isotonic). It is also soluble in 0.02 molar NaCl, in which it is no longer very viscous and no longer shows birefringence of flow. When precipitated (by isotonic NaCl) it does so in strikingly fibrous form. It has the characteristic nucleic acid absorption band, 2540 Å. One-third to one-fourth of the desoxyribose nucleic acid in liver cells has been isolated in this form. In the above properties the liver nucleoprotein is comparable with the nucleohistone from the thymus, which has long been generally accepted as of nuclear origin. The nuclear source of the nucleohistone has been demonstrated cytologically by study of extracted frozen sections of liver, kidney, and pancreas. Upon brief (5–10 minutes) treatment with 1.0 M NaCl the nuclei swell, and lose nearly all their capacity for a positive Feulgen reaction. Isotonic NaCl (5 minutes) shrinks the nuclei, and restores the stainability, though not the original nuclear pattern. Longer treatment with 1.0 M NaCl causes the nuclear contents to flow out of the cell. Isotonic NaCl precipitates this extracellular nucleohistone as long fibres that are Feulgen positive. The chemical and cytological

evidence therefore agree in showing that a desoxyribose nucleoprotein has been extracted from the nucleus.

POWER, MAXWELL E., Yale University, New Haven, Conn.: *Neurological effects of mutants reducing facet number in the eyes of Drosophila melanogaster.*——A number of mutants of *Drosophila melanogaster* which affect facet number have been studied to discover whether the reduction in peripheral sense organs is accompanied by corresponding changes in the central nervous system. The brains of the following stocks have been studied in Bodian impregnated sections: wild type (Oregon R, 780 facets), heterozygous Bar females (358 facets), Bar males (91 facets), Bar females (68 facets), double Bar males (29 facets), and individuals of "eyeless-2" which have no facets. The matrix of the external optic glomerulus is reduced in proportion to the decrease in facet number, because it is composed of centripetal fibers from the ommatidia. A definite volumetric hypoplasia of the middle optic glomerulus has been found which follows the decrease in eye size. This body, however, does not entirely disappear even in completely eyeless individuals. RICHARDS and FURROW (1925) reported similar observations in an anatomical study of the eye and brain of "eyeless." It appears that the centripetal fibers from the eye are necessary for producing the orderly arrangement of the fibrillar components within the middle glomerulus because in the flies from the lower end of the series the characteristic striate appearance of this body is lost. Of the three optic glomeruli the two parts of the inner one are least changed. The several bundles of fibers which connect the middle and inner glomeruli with those glomeruli of the opposite side and with other parts of the brain are constant throughout the series of stocks. The other discrete parts of the brain, *e.g.*, corpora pedunculata, corpus centralis, and protocerebral bridge, remain unchanged.

POWERS, E. L., JR., Johns Hopkins University, Baltimore, Md.: *The determination of mating type of double animals in the ciliate protozoan Euplotes patella.*——With the use of the double animal in *Euplotes patella* which contains two nuclear complexes in a common cytoplasm the following observations have been made. 1) The mating type of a double animal containing but one kind of gene for mating type in one of its nuclear complexes and only one other kind in the second complex is the same as that possessed by a single animal which contains these two genes in its single nuclear complex; that is, no change in mating type is observed when the two different alleles determining the type in question are separated into different nuclei in the same cytoplasm. 2) There is no preferential mating, having its basis in the genes for mating type, of singles with particular halves of these doubles. Each half of the double, therefore, is seen to be equally affected by the genes for mating type in each of the two nuclear complexes present. 3) Because the mating types of these double animals are not affected by the loss of one of their micronuclei, it is concluded that the macronucleus is capable of determining mating type in the absence

of its corresponding micronucleus. 4) Single pronuclei are capable of giving rise to physiologically functional nuclear complexes. 5) Double animals which contain but one kind of allele induce, usually, very little mating in singles heterozygous for that allele. 6) KIMBALL'S hypothesis of genic determination of mating type in this species is supported by the observations made on the inheritance of mating type in these experiments.

POWERS, LEROY, U. S. Department of Agriculture, Horticultural Field Station, Cheyenne, Wyo.: *The nature of the series of environmental variances and the estimation of the genetic variances and the geometric means in crosses involving species of Lycopersicon.*——Weights of individual fruits were obtained from a cross of the Danmark (*Lycopersicon esculentum* Mill.) and the Red Currant (*L. pimpinellifolium* (Jusl.) Mill.). A graph is given that shows the percent of genetic variance included in the variance for between means of blocks when the number of individuals grown per block varies. The experimental design was that of a randomized block. From the graph the number of individuals necessary per block to attain a certain degree of accuracy can be determined. The environmental variances were found to be functions of the means and were found to form a geometric progression. Information as to the nature of the series of environmental variances is essential to the development of methods of separating the genetic from the environmental variation. A method of separating that portion of the variance due to segregation of the genes from that portion of the variance due to environmental influences is presented. The method as it is extended to apply to other data should prove of considerable value in attacking the difficult problem of quantitative inheritance. A method of using the genetic variance to obtain a correction factor to be subtracted from the theoretical geometric mean used in studying the nature of the interactions of the genes affecting weight of fruit was developed and is illustrated. After the correction factors had been applied to the data for the Danmark × Red Currant cross it was found that the data are in agreement with the hypothesis that the effects of the genes differentiating weight of fruit are geometrically cumulative.

QUISSENBERRY, J. H., and BROWN, S. O., Agricultural and Mechanical College of Texas; College Station, Texas: *Inheritance of an eye anomaly in the albino rat.*——Expression of this character is extremely variable. Degrees of anomaly extend from a slight reduction in the size of one eye to the complete absence of both eyes. F_1 and reciprocal backcross progenies including 830 rats were studied. At least one major and one or more minor modifying factors determine the expression of this character. Significantly more females than males are affected. The left eye was more often affected, or affected to a greater degree, than the right eye. It was suspected that certain environmental factors like ration were causing part of the variability in the expression of this character. Responses to rations free of vitamin A and B_1 have been checked. The affected strain was equal to or better than a non-affected strain with

which it was compared in terms of storage and utilization of these vitamins. Embryological development of this anomaly is being studied histologically.

RANDOLPH, L. F., U. S. Department of Agriculture and Cornell University, Ithaca, N. Y.: *The influence of heterozygosis on fertility and vigor in autotetraploid maize.*——Pronounced decreases in vigor and fertility accompany chromosome doubling in inbred strains of maize, while tetraploids derived from stocks that have not been inbred are as vigorous or more vigorous than the diploid parent, and are highly fertile. Tetraploid plants produced directly from inbred lines derived from various commercial varieties, including Luces Favorite, Webbers Dent, Onondaga White Dent, Bloody Butcher, Spanish Flint, the corn belt lines 4-8d, Hy, 187-2, 38-11 and inbred genetic stocks were consistently much shorter and less robust than their diploid sibs. They ordinarily produced few viable seeds, although abundant pollen and well-developed ear shoots were formed. With the single exception of the line 4-8d, repeated attempts to establish tetraploid lines directly from diploid inbreds have been unsuccessful. In contrast to the pronounced deleterious effects of doubling homozygous inbred lines the doubling of heterozygous open-pollinated varieties, or hybrids derived from inbred lines, produced vigorous robust and highly fertile tetraploids. During the inbreeding process in both the diploid and the tetraploid reduction in vigor progresses at essentially the same rate as reduction in heterozygosity, and renewed vigor accompanies the recovery of heterozygosity through the hybrid recombination of genetically dissimilar inbreds. The deleterious effect of doubling the number of homozygous genes at each locus in the tetraploid derived directly from the inbreds and the absence of this effect in the heterozygous tetraploids suggests that homozygosity *per se* is a significant cause of the reduction in vigor which accompanies inbreeding and, conversely, heterozygosity in itself may be responsible for much of the vigor or heterosis exhibited by hybrids.

REED, S. C., CHADWICK, L. E., and WILLIAMS, C. M., Harvard University, Cambridge, Mass.: *Frequency of wing-beat as a character for separating species, races and geographic varieties of Drosophila.*——The Edgerton stroboscope provides a precise method for determining the number of wing-beats per minute of strains of Drosophila. Under standard conditions each species has a characteristic range of wing-beat frequencies. Though the average difference between various strains of Race A and Race B of *D. pseudoobscura* is only 470 double wing-beats per minute, this difference is 7.34 times its standard deviation. Of the several geographic varieties of *D. pseudoobscura* tested, the fastest of Race A flew at 11,470 beats per minute and the slowest of Race B at 10,140; this wide range of frequencies allows one to distinguish physiologically different geographic strains with ease. Reciprocal hybrids and backcrosses to both fast and slow parental strains were secured and showed definitely that the wing-beat frequency is under exact genetic control and that the number of factor pairs concerned is probably not large. The differences in wing-beat frequency

between species, races and geographic varieties were found to rest in large part upon a striking morphological relationship. This relationship is between the muscle-mass of the thorax which is responsible for wing motion and the area of the wings. If the muscle-mass be held constant, the frequency of wing-beats increases uniformly as the wing area, and hence the air resistance, decreases. The genetic changes which have separated strains of Drosophila in regard to the "physiological" wing-beat character are therefore gene changes affecting primarily the proportional relations between wing muscle and wing area.

RICK, CHARLES M., University of California, Davis, Calif.: *Cytological irregularities induced in Petunia by X-ray treatment of pollen.*——Pollen of diploid Petunia was subjected to X-ray doses varying from 10,000 *r* to 100,000 *r* and was subsequently applied to stigmas of untreated plants. All seed produced by pollen surviving the higher doses was germinated on nutrient agar under aseptic conditions. Pollen which had been exposed to doses as high as 50,000 *r* functioned to produce viable seed. No haploid plants were obtained from any of the treatments. Thirty-three plants were found to have gross chromosomal deviations including inversion, translocation, duplication and deficiency. One-third were chimeras in which the chromosome condition of pollen mother cells differed, usually in the presence or absence of a fragment chromosome, from that of the roots. Loss of the fragment in the earliest divisions of the zygote presumably accounts for these differences. Two monosomics were of special interest. In one the odd chromosome nearly always appeared as a univalent, which usually failed to be included in either telophase nucleus in the first meiotic division and appeared as a micronucleus in later stages. In the other monosomic the odd chromosome was associated in a trivalent in the majority of figures and never lagged at anaphase of the first or second divisions. This unusual pairing is attributed either to a duplication present in the normal chromosome complement of Petunia or possibly to a translocation induced by the X-ray treatment.

RIFE, DAVID C., Ohio State University, Columbus, Ohio: *Handedness and dermatoglyphics in twins.*——Comparisons of the occurrence of finger and palm patterns in 740 left-handers with their occurrence in 600 right-handers reveal that bimanual variations in the incidence of single patterns are greater in right-handers than in left-handers. The tendency is much more marked in females than in males. Comparison of the occurrence of finger and palm patterns in 365 sets of twins shows the same type of variation to exist between pairs composed of two right-handers and those consisting of one right and one left-hander, or of two left-handers. The differences are quite marked in female identicals, and in both male and female fraternals. In the identical pairs consisting of one right-hander and one left-hander, the right-handers show as marked left-handed tendencies as do the left-handers. Mirror-imaging of the patterns occurs with approximately the same frequencies in both types of

twins, and shows no correiation with handedness. Other investigations have revealed that twins showing intra-pair differences in handedness are about twice as likely to have left-handed relatives as are those pairs consisting of two right-handers. These observations indicate that whether members of twin pairs have the same or different handedness depends upon the genotype of the twins. (This investigation was made possible by a grant from the National Research Council).

RIDDLE, OSCAR, DUNHAM, H. H., and SCHOOLEY, J. P., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Genetic hermaphroditism in a strain of pigeons.*——In a strain of common pigeons a true hermaphrodite capable of very limited breeding (as a male) was obtained. A normal sister of this hermaphrodite, when outcrossed to a male of another race produced (1st generation) one true hermaphrodite which, when bred as a male to a sister, threw seven true hermaphrodites (2nd generation). Further breeding is already known to have yielded three true hermaphrodites in the third and three in the fourth generations. Offspring in all generations are of the following types: normal females, normal males, males with left oviducts, and males (true hermaphrodites) with ovo-testis and oviduct on the left side and a testis on the right side. The ovo-testes have been found in birds whose ages vary between hatching and 75 months. Biopsy proved the persistence of an ovo-testis for more than 40 months. Of special interest is a fairly large group of males having a left oviduct and two testes without tissue histologically identifiable as ovarian. Such oviducts have been observed to attain a weight of 1.9 grams, which is twice that of the oviduct of a normal female not actively laying eggs. This development of oviducts in birds otherwise apparently male indicates that these birds are producing large amounts of estrogenic hormone and thus, on the basis of their internal secretions, their gonads also are partly female and bisexual.

RILEY, H. P., University of Washington, Seattle, Wash.: *Chromosomal behavior in two natural Tradescantia triploid hybrids with centric fragments.*——Two triploids from a woods near Michaud, Louisiana, showed characters of both *T. paludosa* and *T. canaliculata*. Each had 18 chromosomes plus fragments. At first metaphase the 18 chromosomes showed a high percent of trivalents, bivalents, and univalents and an occasional quadrivalent or hexavalent. Consequently the plants were over 90 percent sterile. In microspores with more than six chromosomes some somatic association of bivalents was observed at metaphase. One plant showed three small, centric fragments in root tip mitosis. These were double at metaphase and lay on the equatorial plate with the large chromosomes which they resembled except for size. At metaphase of the first meiotic division two fragments were paired while the third behaved as a univalent. The second plant had six centric fragments in the root tip. These all lay on the equatorial plate and the daughter fragments passed regularly to opposite poles at anaphase. At first meiotic metaphase

there were three fragment-bivalents normally and two or all three frequently lay alongside one another in a very strong secondary association. The members of the bivalents separated to opposite poles at anaphase. These fragments probably entered the hybrids from the *paludosa* parent as fragments are frequently found in that species. This second plant is triploid in number of large chromosomes and tetraploid in number of centromeres.

RISMAN, G. C., Brown University, Providence, R. I.: *Cell size in Habrobracon.*——Ever since HERTWIG's "Kernplasma" theory was enunciated, studies on the relationship of cell size to chromosome number have been made. In most cases, including both plant and animal material, this size-relation has been shown to hold. In Habrobracon, however, this correlation does not seem to be completely valid. Using haploid males (parthenogenetic, n = 10) diploid males (n = 20) and diploid females (n = 20), the size of the facets of the compound eye was determined. Since each facet is the secretory product of the two underlying cells, statistical differences in size must have some genetic control. The mean measurement across five consecutive facets was found to be: 1n males, 40.43 ± 6.86 mm; 2n males 44.46 ± 7.09 mm; 2n females 40.26 ± 5.72 mm as measured after camera lucida projection. These data corroborate a previous examination of the wing cell size by Speicher, in which the relationship was the same: 1n males = 2n females < 2n males. It would seem, therefore, that some other agent besides mere chromosome number is active in controlling the size of the cell.

ROBERTS, LEWIS M., Connecticut Agricultural Experiment Station and Yale University, New Haven, Conn.: *The effects of translocations on growth in Zea mays.*——This experiment was designed to determine if position effects of rearranged chromosomes can be detected in inbred material especially favorable for the measurement of small phenotypic changes. The obscuring effect of genetic segregation was removed by eight generations of self fertilization. Pollen of the inbred line was irradiated with 1000 *r* units, and from this treatment, 13 reciprocal translocations were cytologically identified. All 10 chromosomes were involved except 7 and 9. Homozygous and heterozygous progenies of the 13 translocations along with the untreated normal inbred from which they came were grown in a replicated field test. No striking differences appeared. Significant small differences, although relatively infrequent, were found by a statistical study of such characters as time of flowering, height, diameter of stalk, width and length of leaf. Changes of a progressive nature were few, but evidence was obtained that they did occur. One homozygous translocation involving three chromosomes averaged two days earlier in shedding its pollen and had a significantly larger stalk diameter than the untreated normal. Significant differences of an adverse effect outnumbered those of a progressive nature in the ratio of approximately five to one.

ROBERTSON, G. G., Yale University, New Haven, Conn.: *Increased viability*

of homozygous yellow mouse embryos in new uterine environments.——Homozygous yellow mouse embryos develop normally through cleavage and blastodermic vesicle formation (contrary to the observations of KIRKHAM, 1919). Just after normal embryos have begun to implant, the homozygous yellow embryo appears as a small mass of vacuolated cells in a decidual crypt in which the uterine epithelium has not disappeared, although the uterus is in the progestational phase. Death of the homozygous yellow embryos occurs after the blastocyst has come in contact with the uterine epithelium, and disintegration and resorption are completed within thirty hours. These abnormal embryos appear in the Mendelian ratio of one abnormal to three normal. The successful transplantation of ovaries from heterozygous yellow mice to homozygous agouti mice has made possible the study of the effects of a new uterine environment upon the development of homozygous yellow mouse embryos. In the agouti uterus these embryos develop further than in the heterozygous yellow uterus. This is indicated by the occurrence of twice the number of cells, by the development of a small ectoplacental cone and Reichert's membrane, and by the disintegration of the decidual epithelium associated with a more advanced development of the implantation site. From this it is evident that the viability of the homozygous yellow embryo is influenced by uterine as well as chromosomal factors.

ROMAN, HERSCHEL, University of Missouri, Columbia, Mo.: *Translocations involving "B" chromosomes in maize.*——"B" chromosomes are supernumerary chromosomes which consist largely of pycnotic material and are nearly or quite devoid of genetic effect. Translocations between these and the regular ("A") chromosomes are of value in connection with various problems concerning the role of heterochromatin in maize, and in providing material for the extraction of specific duplications and for various cytogenetic problems.——Seven translocations between "A" and "B" chromosomes and one between "B" chromosomes are described. All were produced by X-ray treatment of pollen of "B"-bearing plants.——The points of breakage in the "B" chromosome were in both the pycnotic and chromatic regions. The breakage points in "A" chromosomes were in various chromosomes and regions.——The translocations in which the point of breakage in the "A" chromosome has been determined are T1-B, T2-B, T4-B, and T7-B. In the first three the break occurred in the short arm and near the centromere of chromosomes 1, 2, and 4, respectively. In T7-B the break was within a few chromomeres of the end of the long arm of chromosome 7. T2-B, T4-B, and T7-B have been made homozygous.——Duplications of the new chromosome which includes the acentric segment of "A" have been obtained in T2-B and T7-B, in the latter case in homozygous condition.

ROSENBLAD, L. E., University of Houston, Houston, Texas: *A study of relationship between ventral receptacle and testes coiling in Drosophila.*——An analysis of the reproductive tract structures of numerous species of Drosophila

and closely related genera shows a high correlation between the number of ventral receptacle coils in the female and the number of testes coils in the male of the respective species. The ventral receptacle serves as a storage space for the spermatozoa which are produced in the testes. This seems to indicate that there is a need for more coils in the female to take care of the additional spermatozoa produced in the males having a greater amount of gonad tissue. A study of the size relationship between the testes and the spermathecae (also spermatozoa storage organs) in the female shows a positive correlation, but not as high as that between testes and ventral receptacle size. Species which are closely related, particularly those which hybridize, have almost the same number of testes coils and the same number of ventral receptacle coils. The relationship between the number of ventral receptacle coils and the time which the female retains the spermatozoa after fertilization seems to indicate that those species having longer coils retain the sperm longer; this evidence cannot be considered conclusive as other factors such as the retention of the spermatozoa in the spermathecae and uterus may affect the results. The dissection methods employed in this study were the same as described by the author in previous studies.

SANSOME, EVA R., SATINA, SOPHIA, and BLAKESLEE, A. F., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Crossability between species and between tetraploids and diploids in Datura.*——Pollinations of *D. stramonium* by *D. metel* result in abundant fertilizations but in no viable seeds. Abortion of proembryo and endosperm occurs when they have formed 4 to 8 cells and about 100 cells respectively. Abortion has been assumed to be due to incompatibility between genes brought into the hybrid from the two parental species. A similar abortion occurs in most ovules from tetraploid by diploid pollinations within a highly inbred line, hence differences in genes cannot be the sole cause of embryo abortion. Since pollen tubes from a tetraploid usually burst in 2n styles, pollinations of 2n×4n generally fail to set capsules. By availing ourselves of the fact that the inside of the style through which pollen tubes grow is of epidermal origin, it is possible to make the cross 2n×4n by means of a 4n, 2n, 2n periclinal chimera. This cross results in a similar abortion after slight growth of embryo and endosperm. Since the ratios of chromosome numbers between embryo, endosperm and female somatic tissue in the 4n×2n cross deviate in opposite directions from those in the 2n×4n cross in comparison with the compatible crosses 2n×2n and 4n×4n, it is difficult to explain the abortion on the basis of a simple relation between the balanced chromosome numbers. In the three crosses mentioned, the abortion is associated with pseudoembryo formation which can also be stimulated by auxin injection. Some relation to chemical regulation is therefore suggested.

SHULL, GEORGE H., Princeton University, Princeton, N. J.: *Oenothera seg. acuminata, Oe. seg. contracta, and other mutational segregations from Oe. Lamarckiana and Oe. mut. erythrina.*——Repeatable mutational segregations have

been found in cultures of *Oe. Lamarckiana* and its derivative, *Oe.* mut. *erythrina*. These differ from the usual mutations from these forms, in being produced repeatedly on selfing some or all plants of the phenotypically normal parental type. Most of these mutational segregates have not bloomed, and many of them are so weak or of such slow growth that they probably will never bloom. The demonstration consists of photographs of the mutational segregates in contrast with their parents, with special emphasis on *Oe.* seg. *acuminata* from *Oe. Lamarckiana*, and *Oe.* seg. *contracta* from *Oe.* mut. *erythrina* which have been brought to bloom by means of special handling. The mutational segregations from *Oe.* mut. *erythrina* are of two kinds: (a) those which replace *Oe.* seg. *decipiens*, or (b) those which are additional to *Oe.* seg. *decipiens*, or to the segregate which has been substituted for seg. *decipiens*.

SONNEBORN, T. M., Indiana University, Bloomington, Ind.: *Inheritance of an environmental effect in Paramecium aurelia, variety 1, and its significance.*——In stocks homozygous for the gene $+^{II}$, crosses of mating type I × mating type II yield some clones of each type, with the percentage of type II clones $= 18.4 + t/0.51$, where t is the temperature during conjugation and nuclear reorganization. This relation holds through the range 10° to 35°C., and is based on a study of 1760 clones obtained at 9 different temperatures. After nuclear reorganization is completed, the mating type is not altered by temperature or other environmental conditions; it is inherited indefinitely through reproduction by fissions. These relations, although similar to some that have been interpreted as Dauermodifikationen, are not in conformity with this concept. Their interpretation is suggested by the two following facts. 1) Differences in mating type are determined by differences in the macronuclei. 2) Identical homozygous micronuclei give rise to diverse macronuclei. These macronuclear diversities, determining hereditary characters, are therefore mutations of some kind. The simplest interpretation seems to be that the $+^{II}$ gene is for type I and mutates with high frequency to an allele for type II, though only at the time the macronuclei develop from micronuclei. The high mutation rate, 38 percent to 87 percent as temperature rises from 10° to 35°, is enormously greater than any hitherto recorded. The randomness of occurrence of the changes is typical of mutations, for the two macronuclei that develop in the same reorganizing cell show independence and randomness in their determination.

SPARROW, A. H., McGill University, Montreal, Canada: *Spiralization in microspore chromosomes of Trillium.*——At metaphase and anaphase of microspore mitoses the chromonemata form a large-gyred hollow spiral. Along its gyres an irregular waviness or "minor" somatic spiral is visible. The spiral develops during prophase by an increase in gyre size and a decrease in gyre number and in chromatid length. Chromosome volume and chromonema (not chromatid) length both increase significantly between early metaphase and anaphase. Where the spiral can be seen to be double-stranded it is plectonemic.

The process of reducing this plectonemic spiral into parallel, freely-separable chromatids begins in one prophase as a reduction in gyre number and continues as relational uncoiling in the next. Paradoxically, therefore, a spiralization cycle such as that described above can be interpreted as an uncoiling process in which successive cycles overlap in prophase.

SPARROW, A. H., RUTTLE, MABEL L., and NEBEL, B. R., N. Y. State Agricultural Experiment Station, Geneva, N. Y.: *Sterility differences between auto- and allotetraploid Antirrhinum.*——Autotetraploids of 15 varieties have been produced by treatment of young seedlings with 0.16 percent colchicine. These plants set very few seeds and have, with the exception of one variety, continued highly sterile in the second and third generations. Allotetraploids were obtained by crossing the different varieties of autotetraploids. They set an abundance of seed. The marked difference in fertility of the two types of tetraploids cannot be correlated with differences in the number of quadrivalents at first metaphase nor to the relative proportions of good and bad pollen in acetocarmine smears. Thus in both the auto- and allotetraploids so far examined four to six quadrivalents were most frequently observed, but cells with higher and lower numbers of quadrivalents also occur. In an allotriploid examined the number of trivalents ranged from two to eight. Lagging and loss of chromosomes at first and second anaphase result in a variable amount of pollen abortion and numerous microcytes. In autotetraploids the amount of good pollen grains in different populations ranges from two to three percent up to about 95 percent, but is in no case correlated with very high fertility. In first generation allotetraploids the number of good pollen grains ranges from 35 to 95 percent and in all cases the plants set an abundance of good seed. Thus it appears that the sterility differences in the two types of tetraploids cannot be attributed merely to meiotic irregularities nor to pollen deficiencies.

SPENCER, W. P., College of Wooster, Wooster, Ohio: *Inherited variations in wild populations of Claytonia virginica.*——In extensive populations of *Claytonia virginica* in Wayne County, Ohio, two widely spread alternate types of sepal color, green and brown, have been observed. Only the green sepal type is found in the upper part of Little Apple Creek drainage system and in the entire Christmas Run drainage. In other regions small local colonies of several hundred plants exclusively brown occur. The Killbuck and Clear Creek valleys show mixed populations of green and brown with small local areas of one or the other. The hybrid between these two color types is gray. Green sepals streaked with purple is a relatively rare variant observed in several localities. In a low, swamp forest a colony was found with plants having green sepals with purple tips, changing to deep reddish-purple sepals as the flower aged. A small colony with petal number reduced has been located in Little Apple Creek valley. Fused inflorescence, in which the individual flower pedicels are united, has been found in several places. There are also variant types of petal size, shape, and color, and of sepal size and shape. These variants are actually mutant types

and not environmental effects as shown by the following facts: (a) the position and extent of colonies of certain variants have been observed in two successive years, 1940 and 1941; (b) tubers have been transplanted and have given the same variant type in the new environment; (c) all of the stems and flowers from a single tuber show the distinct variant in question. Owing to large numbers of individuals, extent of populations, and variety of habitats occupied this plants offers unusual opportunities for studies in micro-evolution.

SPENCER, W. P., College of Wooster, Wooster, Ohio: *Are natural mutations in wild Drosophila distributed in a Poisson series?*——In extensive inbreeding tests of wild *Drosophila hydei* populations it has been established that approximately one visible mutant is present in heterozygous form per four flies tested (exclusive of the special case of bobbed). Similar, but less extensive tests on *D. melanogaster* and *D. robusta* show the former to carry more mutants and the latter less in diverse populations. The findings of several cases of closely linked mutants present in the same wild chromosome raised the question of whether mutants in nature are distributed in a Poisson series among chromatin blocks of 10 units length or less. Basing calculations on the short *melanogaster* map of 280 units per chromosome set (*hydei* maps are longer), there are roughly 56 chromatin blocks of 10 units length per fly, and 224 such blocks per four flies. 264 mutants have been found distributed as follows: 251 chromatin blocks, 1 mutant each; 5 blocks, 2 mutants each; 1 block, 3 mutants. Each of the 257 blocks in which mutants were found represents the testing of about 224 blocks, or in all, the mutants were distributed in a chromatin block population of 57,568 in the manner recorded. Applying the Poisson formula, with grouping together of doubles and triples in the Chi-square calculation, the data clearly indicate that the mutants are not distributed at random to short chromatin blocks. Owing to the operation of decay, we are probably observing only the terminal traces of what was initially a much more marked non-random distribution.

STEINBERG, ARTHUR G., McGill University, Montreal, Canada: *Further studies on the histological development of the wild type and Bar eyes of Drosophila melanogaster.*——It has been reported previously that "cell clusters" consisting of four cells each are present in the eye discs of wild type larvae but not in Bar larvae at 72 and 96 hours of larval development at 25°C (STEINBERG, Genetics **26**: 325–346). These "cell clusters" have now been found to be cross sections through "goblet-like" structures. The latter structures, first observed in prepupae, were subsequently found in both 96 and 72 hour larvae. None were found in 48 hour larvae, thus confirming the earlier observations that cellular organization occurs after 48 hours and prior to 72 hours. The development of the "goblet-like" structures has been followed through the pupal period. *They are the precursors of the ommatidia and develop directly into them.* The "goblet like" structures are present also in both 96 and 72 hour old Bar larvae although in much smaller numbers than in wild type. (For example, no more than 4

goblets were observed in any section of a Bar eye disc while more than 20 have been observed in wild type.) Hence, there is probably no difference between Bar and wild type in the time of onset of the differentiation of the precursors of the ommatidia. These observations lend further support to the hypothesis advanced by STEINBERG (loc. cit. and Genetics **26**: 440–451) in which the difference in facet number between Bar and wild types is ascribed to a difference in the number of cells entering into the formation of the cephalic complex.

STONE, WILSON S., GRIFFEN, A. B., and PATTERSON, J. T. University of Texas, Austin, Texas: *Drosophila montana, a new species of the virilis group.* ——This species belongs to the wild-type forms of the virilis group. Somatic metaphase plates show twelve chromosomes: four pairs of rods, one pair of small V's and one pair of dots. The V-shaped element is the result of a complex rearrangement in chromosome 2, with an insertion of the centromere near the middle of the long euchromatic arm. No fusion of chromosomes is present. Thus far we have been able to hybridize it with *D. texana* and *D. novamexicana.* A study of the salivary chromosomes of the *montana-texana* hybrid larvae shows the following points: The X chromosome is identical with that of *texana;* chromosome 2 has the *texana* inversion and, in addition, the complex transposition of the centromere; chromosome 3 shows two included inversions, neither of which has been found in any of the other forms; chromosomes 4 and 5 show gene orders not yet observed in any other member of the group; finally, both X and 4 may show inversions in hybrids between strains of *montana.*

STURKIE, PAUL D., Alabama Agricultural Experiment Station, Auburn, Ala.: *Suppression of a dominant character, polydactylism, in the domestic fowl.* ——This report concerns the effects of low temperatures on the suppression of polydactylism in embryos, heterozygous and homozygous for the character, at two, three, four and five days of development. The eggs were incubated at the control temperature of 37.5°C for the above periods of time. Each of these age groups of embryos was then subjected to a temperature of 2.5–4°C for a period of 16 hours, then incubated for 2–3 days at about 34°C, after which time they were placed on control temperatures. From a mating of a homozygous polydactylous Houdan male to ten normal White Leghorn females, the eggs from which were incubated at control temperature, 207 polydactylous and two normal chicks were hatched. Embryos from this same mating were subjected to low temperatures at the above mentioned stages. The number of chicks hatched, which had received the cold treatments at two, three, four and five days were respectively 132, 160, 55, and 55. The percentages of non-polydactylous chicks produced from these respective treatments were: 78.8, 56.0, 56.0 and 18.0. The results indicate suppression of the character. The least effective treatment in suppression was that employed at five days of development. In most cases at this stage, the extra hind toe is differentiated, whereas at four days and earlier it is not. When embryos homozygous for polydactylism were subjected to the cold treatments at two, three, and four days, 19 percent of the hatched chicks were non-polydactylous.

VILLEE, CLAUDE A., University of California, Berkeley, Calif.: *The effect of cold treatments upon the development of the mutant aristapedia-Bridges in Drosophila melanogaster.*——Normally in aristapedia-Bridges only the base of the arista is changed to a fleshy lobe, the rest being plumose. Exposing developing larvae to a temperature of 14.4°C produces an increased expression of the character; in extreme instances the phenotype becomes identical with that of aristapedia, *i.e.*, the entire arista becomes tarsus-like and claw-tipped. The leg tarsi are abnormal, reduced in size and in number of segments. Cold treatments were begun 0 to 6 days after the parents were removed and continued one to eight days. A treatment of any given length produces its greatest effect if begun on the fourth day of development. In treatments begun immediately after the parents were removed or on the fifth or sixth day, the duration of the treatment made little difference in the effect. This was also true in treatments begun on the first or second day and ended before the fifth; however, if treatment was continued past the fifth day, the effect increased proportionally with the length of the treatment. Treatments begun on the third or fourth day produced an effect proportional to the duration of the treatment. This indicates that a decrease in the velocity of development produced by cold treatment enables the aristapedia-Bridges gene, working at a lower rate for a longer time, to produce a phenotype similar to aristapedia. Although the effect produced is greatest during the fourth day, some effect results from treatment during any day of development from the first to the sixth, indicating that there is no sharp T. E. P. for the reaction involved.

WALETZKY, E., and OWEN, R., University of North Carolina, Chapel Hill, N. C., and University of Wisconsin, Madison, Wis.: *A case of inherited partial sterility and embryonic mortality in the rat.*——A partially sterile condition, PS, appeared spontaneously in a single male and is inherited by half of his offspring. Although average litter size in the colony is over seven young, PS rats consistently produced about three young per litter when mated to normal rats. Eight out of 16 tested F_1 and F_2 males and females from the original male were PS and eight were normal. Both PS and normal females mated to normal and PS males respectively contain a normal number of implantation sites, but approximately 60 percent of these sites undergo resorption. The implantation sites are all equal in size at 8–9 days after mating, but gradually become markedly unequal. At 14–15 days resorbing sites contain only solid placentomata without any trace of an embryo. From 11 to 14 days, 19 out of 71 resorbing sites contained very small, relatively undifferentiated embryos, and the rest contained solid placentomata. Many of the abnormal embryos were partially or completely necrotic, and a few had open neural tubes anteriorly. At the earliest stage examined, 8–9 days, approximately half the sites contained normal embryos, and the other half solid placentomata with or without disintegrating fragments of an embryo.——The simplest hypothesis which seems to explain this partially sterile condition and the presence of several types of abnormal embryos is a heterozygous reciprocal translocation.

WALTERS, JAMES, L., and STEBBINS, G. LEDYARD, JR., University of California, Berkeley, Calif.: *Distribution of structural hybrids in Paeonia californica.* ——*Paeonia californica*, native to central and southern California, contains structurally homozygous forms, with five pairs at I metaphase, as well as structural heterozygotes showing all possible degrees of heterozygosity, namely ⊙ 4+3 pairs, ⊙ 6+2 pairs, 2⊙'s 4+1 pair, ⊙ 8+1 pair, ⊙ 6+⊙ 4, ⊙ 10. The structurally homozygous forms occupy a large area in the center of the range of the species, but are absent from its southern and northern ends. Within the area occupied by the homozygotes, colonies containing both homozygotes and various types of heterozygotes have been found. No difference could be found between homozygotes and heterozygotes in external morphology. The morphological variability among individuals of a colony appears the same in regions where only heterozygotes occur as it is in regions where both structural homozygotes and heterozygotes are found.

WARMKE, H. E., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *A new method for determining the sex heterozygote in species with morphologically undifferentiated sex chromosomes, and its application to Silene otites.* ——*Silene otites* is dioecious, but whether the male or female is heterogametic (XY) cannot be determined by cytological methods because of similarity in size of the sex chromosomes. By doubling the chromosome number and by utilizing the peculiar breeding behavior of the 4n sex heterozygote, it has been possible to present evidence that the male is heterogametic. The method is as follows: Using colchicine, XX and XY plants are transformed into XXXX and XXYY plants, respectively. The XXXX individuals will produce only XX gametes, but the XXYY individual will produce at least 1 XX:4 XY:1 YY gametes. A higher proportion of XY gametes will result if differentiation of the sex chromosomes is sufficient to disturb random pairing. When XXXX and XXYY individuals are crossed, therefore, three types of offiispring are expected: 1 XXXX:4 XXXY: 1XXYY. In Silene approximately 5 males to 1 female are obtained when treated 4n males and females are crossed, which indicates two classes of males: 1 XXYY and 4 XXXY, or 1 XXXX and 4 XXXY, depending upon whether the female is XXXX or XXYY. To determine the constitution of the female, several of the treated 4n females are crossed to diploid males (and reciprocal if possible). If the female is XXXX, a 3n population of 1 male to 1 female should result; if it is XXYY, one should expect 5 males to 1 female. In Silene this cross has produced triploid males and females in a ratio that does not deviate significantly from 1:1. This indicates that females are homogametic and males are heterogametic, and makes possible the interpretation of breeding results on the same basis as in Melandrium.

WHITING, ANNA R., University of Pennsylvania, Philadelphia, Pa.: *X-ray sensitivity of first meiotic prophase and metaphase in Habrobracon eggs.*—— Hatchability percentages of unfertilized eggs X-rayed in late prophase I with

dosages up to 400 *r* are significantly higher than of controls. Above 400 *r* dosage-hatchability curve indicates that death depends upon the concurrence of two or more events the chance of which increases disproportionately with increased radiation. The lethal dose is about 35,000 *r*. In contrast, dosage-hatchability curve of eggs X-rayed in late metaphase I indicates that death results from one event. It is unchanged by time-intensity differences or by fractionated dosages. These eggs are sensitive to 50 *r* and the lethal dosage is about 1250 *r*. After 1864 *r* all suitable stages show fragments (terminal deletions ?) within the telophase I spindle or between equatorial plates at metaphase II. With 500 *r*, 750 *r* and 1000 r, percentages without visible fragments form a curve parallel to and somewhat higher than dosage-hatchability curve. Mean fragment number per egg increases with dosage. No eggs treated in prophase I with 1864 *r* show fragments. Eggs treated in metaphase I with 2500 *r* pass at once into telophase I when laid (like controls). 97.5 ± 1.35 percent complete meiosis and begin cleavage at approximately normal rates. When egg-laying is temporarily restrained to keep chromosomes relatively inactive after treatment hatchability is not significantly changed. If stresses peculiar to metaphase I chromosomes sensitize this stage to irradiation, treated prophase I chromosomes, which later undergo these stresses, must receive but slight injury at dosages lethal to former or recover almost completely before entering metaphase I. Data indicate that metaphase injury, like prophase, is due to chromosome breaks correlated with ionizations and not to "physiological" causes, stickiness and clumping of chromosomes.

WILSON, G. B., and BOOTHROYD, E. R., McGill University, Montreal, Canada: *Differential reactivity in the chromosomes of Trillium species.*——Rhizomes of *Trillium erectum*, *T. grandiflorum* and *T. undulatum* exposed to a temperature of 3°C show a characteristic differentiation at metaphase and anaphase of root tip chromosomes into deeply and lightly stained regions. The differential pattern is a species characteristic and the three species studied show marked differences in their patterns. The rate with which the chromosomes become affected in *T. erectum* has been considered in some detail. A few regions appear after one hour and an apparent maximum is reached after 96 hours exposure. The same treatment has produced a similar differentiation in the somatic chromosomes of two varieties of rye.

WILSON, G. B., and SPARROW, A. H., McGill University, Montreal, Canada: *Partial fusion of untreated root tip chromosomes of Trillium erectum L.*——In a single root tip of *Trillium erectum* a number of metaphase plates showed one or more pairs of chromosomes which were fused for a short distance. In at least two cases the associated pairs are apparently homologous. In other cases, however, the associated chromosomes are non-homologous. In some cases this fusion involves breakage and rejoining of chromatids resulting in at least one instance in a bridge and fragment at anaphase. Other rhizomes collected at the same time and similarly handled before being examined showed no such irregularities.

ZAMENHOF, S., New York, N. Y.: *Selective irradiation.*——The possibility of ultraviolet irradiation of arbitrarily selected minute areas of chromatin is a step towards realization of selective irradiation of particular genes. Another advantage of experimentation with localized action of U.V. rays is the possibility of using higher dosages without lethal effects. It may be also of interest to irradiate cytoplasm alone instead of nucleus in order to find out whether or not the mutation-causing energy can pass from the cytoplasm to the nucleus. To produce minute spots of U.V. light two methods are feasible. 1) In H. J. MULLER's method the condenser of a microscope is replaced by a short-focal quartz or fluorite lens which produces on the stage of the microscope (or on the living cell to be irradiated) a minute picture of a small and remote opening illuminated by the source of U.V. rays. 2) The author's method uses a screen opaque to U.V. rays but provided with an opening less than 0.5μ in diameter; the screen is placed directly beneath the cell to be irradiated. The author checked experimentally the size of luminous spots obtainable by these two methods. While the diffraction phenomena restrict the lower limit of the size of the irradiated spot, yet a spot as minute as 0.2μ in diameter with the lens method, and 0.9μ in diameter with the screen method, is obtainable.

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